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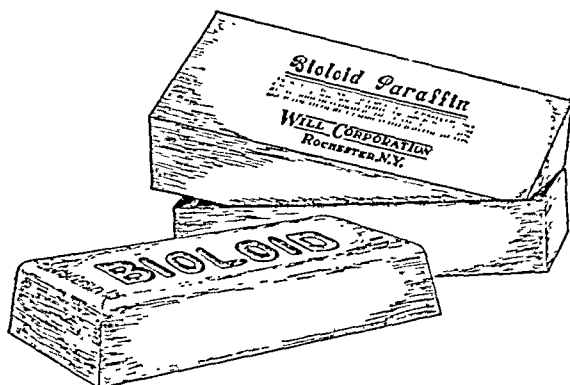
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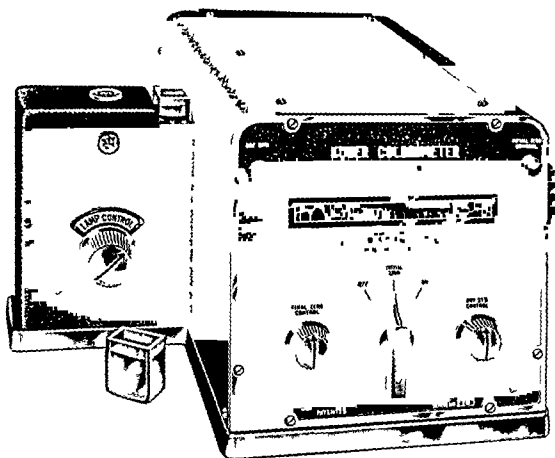
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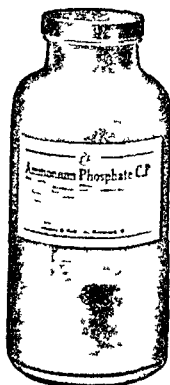
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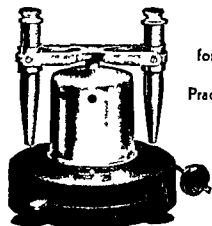
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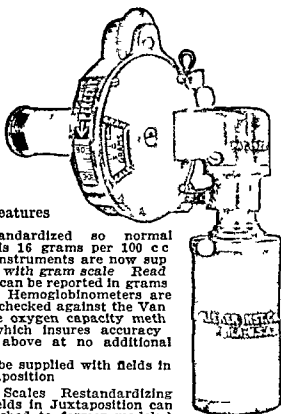
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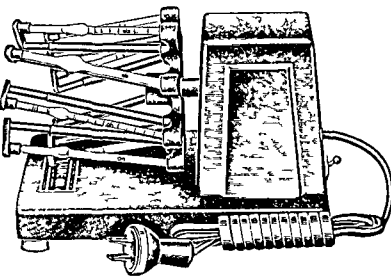
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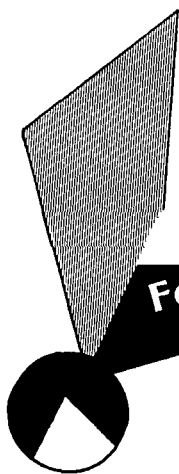
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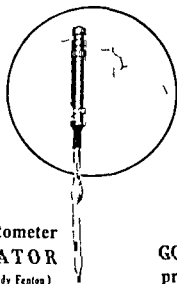
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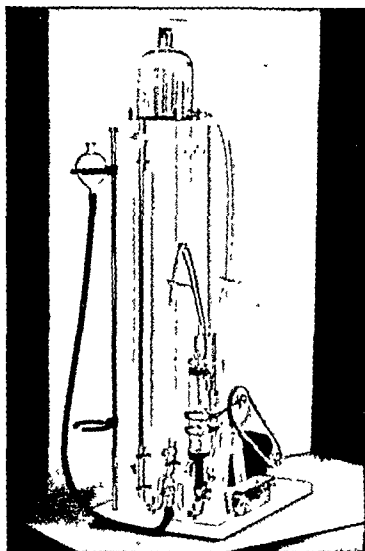
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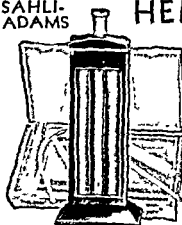
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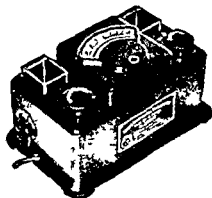
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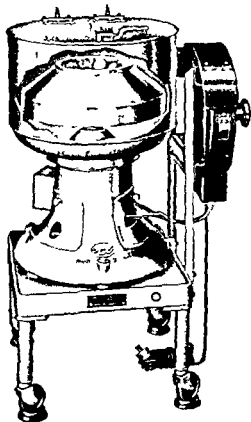
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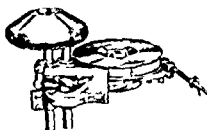
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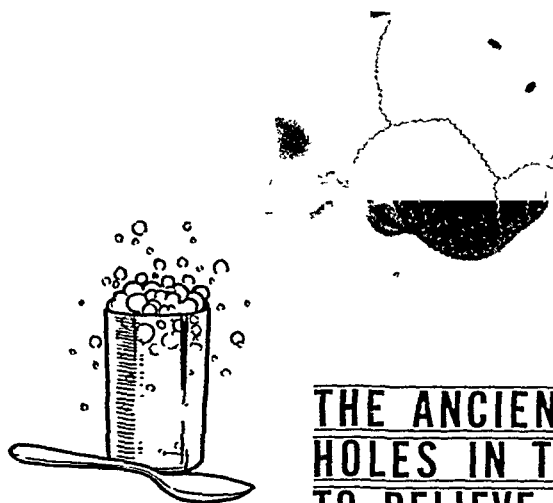
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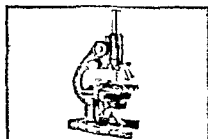
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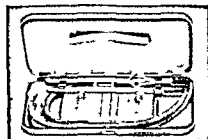
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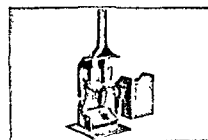
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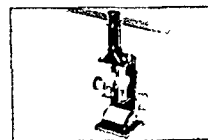


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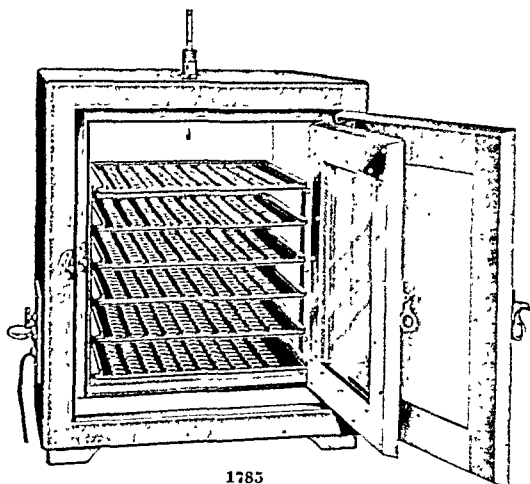
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## CLINICAL AND EXPERIMENTAL

### BACTERIOLOGIC STUDIES OF THE BLOOD OF NORMAL INDIVIDUALS AND RHEUMATIC FEVER PATIENTS\*

ESTHER MEYER, PH D, M S, AND VIRGINIA RYAN, CHICAGO, ILL

THIS work began almost four years ago, soon after Kendall<sup>1</sup> described his new medium. The results reported in this paper deal only with the bacteriologic findings obtained by serial transfer technique. Each blood sample was divided into two parts, one portion was cultured on Kendall's fluid media (E M), the other portion of the same specimen was cultured on dextrose calcium carbonate veal infusion broth (V R). After incubation of these flasks, heavy inocula were transferred to surfaces of lactose agar plates and transferred in series as first described by Hauduroy<sup>2</sup> and modified by Ryan.<sup>3</sup> Bloods of normal children, rheumatic fever children, and normal adults were investigated by this method. There are many reports appearing in the current literature on the bacteriology of rheumatic fever. Various types of dissociated bacteria have been described. It is not possible with our present knowledge to evaluate the significance of these findings in rheumatic fever cases.

Clawson<sup>4</sup> and Callow<sup>5</sup> have reviewed the literature on the bacteriology of the blood in rheumatic fever patients.

*Technic*—After applying tincture of iodine to the arm site and then removing it with 96 per cent alcohol, 10 c.c. of blood was drawn into a sterile syringe. The blood was immediately transferred, using aseptic technique, to a sterile test tube and placed in the ice chest. (All syringes were rolled in towels and autoclaved at 15 pounds' pressure for twenty five to thirty minutes.) The needles were placed in cotton stoppered small tubes and heated in the hot air oven at

\*From the Departments of Bacteriology, University of Illinois Colleges of Medicine and Pharmacy and Research Laboratories of the State Department of Public Health.  
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190° C. for one hour. The syringes were assembled immediately preceding use. After remaining in the ice chest overnight, the serum was removed with a sterile pipette and the remaining clot was then divided as nearly as possible into two equal portions. One part of the serum and clot was in each instance added to 100 c.c. flasks of Kendall and veal infusion dextrose broth media. The Kendall's medium was made up according to the published record.<sup>1</sup> The dextrose carbonate broth was prepared as follows: Five hundred grams of lean veal, 20 gm. of Difco peptone, 5 gm. of sodium chloride, and 1,000 c.c. of distilled water; adjusted to pH 7.8; and calcium carbonate added to each flask (100 c.c.) and then autoclaved. Two cubic centimeters of a 20 per cent Berkefeld filtered dextrose was added to each flask before inoculation, with the clot. All flasks were incubated at 37° C.

A modification of Clawson's<sup>4</sup> technic was used. Gram-stained smears were prepared from each inoculated flask every forty-eight hours; for every two seeded flasks there was one uninoculated 100 c.c. flask control included in our series. In no instance have we found a growth after 20 serial transfers on our control sterile medium. As soon as organisms were detected in the stained films, 0.2 to 0.3 c.c. of the culture was seeded onto a sterile lactose agar plate. Incubation of all other flasks not showing bacterial forms on smears was continued for fourteen days and then the flasks were routinely seeded in the same manner to lactose agar plates. All plates were incubated at 37° C. and examined carefully every twenty-four hours with the low power lens of the microscope. If no growth appeared after forty-eight hours, serial transfers were started, using Ryan's<sup>3</sup> modification of Hauduroy's<sup>2</sup> technic. In the event that no growth occurred after twenty serial transfers on lactose agar, the series was then discontinued and recorded as negative. All lactose agar plates used in this work were incubated overnight and carefully examined for any minute colonies before being used. The rim of the dish was flamed in the Bunsen burner before and after being opened. Only the central portion of each dish was used in spreading the inoculum. Gram-stained films were prepared systematically as soon as growth was observed on the plate. All original flasks were allowed to remain in the incubator from four to six weeks and before being discarded, agar plates were spread, in order to determine whether growth had taken place in the flasks after prolonged incubation.

Space does not permit long detailed protocols, although we realize these would give most information. Previous workers have not reported their results in sufficient detail for us to compare with our observations. We are recording the first five cultures carried out in serial transfer to indicate our findings with each of the three series of bloods examined. We do not see any advantage of Kendall's medium over the dextrose carbonate veal infusion broth. Table IV gives a résumé of our findings. It will be noted that growth occurs using serial transfers after carbonate broth a little sooner than after Kendall's medium. This holds for normal children, rheumatic fever children and normal adults. The material was removed for Kendall's medium study first and then the remaining blood sample used for the dextrose carbonate broth, which most probably explains the higher incidence of contamination in the latter series of tests.

Typical cultures obtained by the serial transfer method from blood of two normal children, one normal young adult, and two rheumatic fever children were selected for some immunologic experiments. Lactose agar plates were seeded and growth washed off with saline. Rabbits were injected intravenously with fresh

TABLE I

RECORD OF BACTERIOLOGIC RESULTS OF BLOOD OF NORMAL ADULTS OBTAINED BY SERIAL TRANSFER METHOD. KENDALL AND CALCIUM CARBONATE MEDIA WERE BOTH USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	8	R	Diphtheroid	2+	Salmon pigment
	CaCO <sub>3</sub>	1	R	Diphtheroid	2+	
2	K	7	R	Diplococci	2+	
	CaCO <sub>3</sub>	4	R	Diplococci	4+	
3	K		--	--	--	Negative up to twentieth serial plate
	CaCO <sub>3</sub>		--	--	-	Negative up to twentieth serial plate
4	K	8	R	Diplococci	2+	Short chains in broth
	CaCO <sub>3</sub>	4	R	Diplococci	4+	
5	K	10	R	Minute diphtheroids	3+	Short chains and irregular groups in broth
	CaCO <sub>3</sub>	4	R	Diphtheroids	3+	

TABLE II

BACTERIOLOGIC FINDINGS BY SERIAL TRANSFER METHOD OF BLOOD OF NORMAL CHILDREN. KENDALL'S MEDIUM AND CALCIUM CARBONATE BROTH WERE USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	6	R	Diphtheroids	2+	Orange pigment on ageing
	CaCO <sub>3</sub>	1	R	Diphtheroids	1+	
2	K	1	R	Diphtheroids	2+	
	CaCO <sub>3</sub>	4	R	Diplococci Diphtheroids	1+	
3	K	5	R	Diphtheroids	2+	Rose pigment after several transfers
	CaCO <sub>3</sub>	5	R	Diphtheroids	1+	
4	K	9	R	Slender diphtheroids	1+	
	CaCO <sub>3</sub>	3	R	Diplococci diphtheroids	1+	
5	K	4	R	Gram + bloated nodular rods	1+	
	CaCO <sub>3</sub>	5	R	Large diphtheroids	1+	

living suspensions twice weekly for five weeks, 1 and 2 cc of the suspensions were injected. Trial bleedings were made after the fifth injection. One week after the last injection animals were bled from the carotid artery. No detectable reaction occurred during course of injection in rabbits. No agglutinins were demonstrable in any antisera tested at any time. There were no pathologic lesions in rabbits at autopsy after carotid bleeding. The bacterial suspensions were used as antigens with sera of the same subject, no agglutinins could be demonstrated.

We were unable to find significant differences between the bacterial flora of the bloods of normal and rheumatic fever children and normal adults using serial transfer technic. All of these specimens would be considered sterile by the usual diagnostic laboratory methods.

TABLE III

BACTERIOLOGIC STUDIES OF BLOOD OF RHEUMATIC FEVER CHILDREN BY SERIAL TRANSFER METHOD. KENDALL'S MEDIUM AND CALCIUM CARBONATE BROTH WERE USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	4	S	Diphtheroids	1+	Yellow pigment after two transfers on Lac. agar; then lost pigment
	CaCO <sub>3</sub>	5	R	Diphtheroids	1+	
2	K	3	R	Diplococci diphtheroids	1+	
	CaCO <sub>3</sub>	3	R	Diplococci diphtheroids	1+	
3	K	3	R	Gram-positive diplococci	3+	
	CaCO <sub>3</sub>	3	S	Giant cocci	3+	
4	K	6	S	Diphtheroids	1+	
	CaCO <sub>3</sub>	3	R	Diphtheroids	1+	
5	K	3	R	Diphtheroids	1+	
	CaCO <sub>3</sub>	3	R	Diphtheroids	1+	

TABLE IV

RÉSUMÉ TABLE OF SERIAL TRANSFER RESULTS OBTAINED FROM BLOOD OF NORMAL CHILDREN AND ADULTS AND OF RHEUMATIC FEVER CHILDREN

CULTURES	CHILDREN				NORMAL ADULTS	
	NORMAL		RHEUMATIC			
	"K"	CaCO <sub>3</sub>	"K"	CaCO <sub>3</sub>	"K"	CaCO <sub>3</sub>
Average Serial transfer on which growth appeared	8.6	6.0	6.1	5.0	7.5	5.2
"Sterile"	3	2	0	0	5	2
Negative after twenty transfers						
Contamination	2	3	3	6	4	9

## REFERENCES

1. Kendall, A. I.: Observations Upon the Filtrability of Bacteria Including a Filterable Organism Obtained From Cases of Influenza, *Northwestern Univ. Bull., Med. School* 32: 1, 1931.
2. Hauduroy, P.: Techniques de Culture des Formes Filtrantes Invisibles des Microbes Visibles, *Compt. rend. Soc. de biol.* 97: 1392, 1927.
3. Ryan, V., and Arnold, L.: Dissociation of Yeast and Bacteria Within the Stomach and Duodenum, *Proc. Soc. Exper. Biol. & Med.* 29: 899, 1932.
4. Clawson, B. J.: Studies on the Etiology of Acute Rheumatic Fever, *J. Infect. Dis.* 36: 444, 1925.
5. Callow, B. R.: Bacteriologic Investigation of the Blood in Rheumatic Fever, *J. Infect. Dis.* 52: 280, 1933.

## XYLOSE TOLERANCE OF RABBITS WITH URANIUM NEPHRITIS\*

HARDY W. LARSON, PH D, NEW YORK, N Y

THE clearances of ingested foreign sugars have assumed considerable importance with their use as kidney and liver function tests. This investigation is concerned with xylose tolerance of normal rabbits and of rabbits with uranium induced nephritis. Since it has been claimed<sup>1,3</sup> that xylose clearance is a delicate index of renal function, it was presumed that the impairment of function caused by the injection of very small quantities of uranium acetate would be indicated by a slight lowering of the xylose tolerance. The results of the administration of these small doses are of interest from the standpoint of both xylose tolerance and uranium intoxication. A perusal of the voluminous literature on uranium poisoning has yielded little concerning the effect on the kidneys of very small quantities of this toxic substance, or the progressive effect and the degree of uranium resistance developed when repeated and increasing small doses are given.

### EXPERIMENTAL

Rabbits varying in age from one and one half to four years were used for the experiment. A preliminary xylose tolerance test performed on each animal indicated normal kidney function. The animals were given, without preliminary fasting, 1 gm of xylose per kilo body weight by stomach tube, 50 per cent sugar solutions being administered and washed down with 25 cc H<sub>2</sub>O. The non fermentable sugar of the blood was then followed for five hours. The rabbits were bled before the sugar was given and at hourly intervals thereafter the blood being obtained from the marginal vein of the ear. A 0.2 cc blood sample was laked in 3.8 cc H<sub>2</sub>O in a centrifuge tube, 1 cc of a 25 per cent suspension of yeast (washed three times with H<sub>2</sub>O) added, and the tubes allowed to stand one half hour at room temperature. Five cubic centimeters of tungstic acid made from equal parts of H<sub>2</sub>SO<sub>4</sub> (120 cc of 0.666 N H<sub>2</sub>SO<sub>4</sub> in 1 liter) and sodium tungstate (120 cc of 10 per cent Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O in 1 liter) were then added, the tubes shaken and centrifuged. Five cubic centimeters of the tungstic acid filtrate were used for the nonfermentable sugar determination by the method of Folin and Malmros.<sup>4</sup> Urea nitrogen determinations on 0.2 cc blood were made simultaneously with the xylose tests using the manometric method of Van Slyke.<sup>5</sup> Uranium acetate in amounts varying from 0.2 mg to 1 mg per kilo was injected subcutaneously, and the xylose tolerance run two to four days after injection, or in some cases the sugar tolerance was determined daily after injection in order to ascertain the period of maximum effect. For purposes of

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studying the effect of liver function on the clearance of xylose, a few experiments were undertaken in which the rabbits were poisoned by subcutaneous injection of phosphorus in olive oil in amounts varying from 0.4 to 1 mg. per kilo. In the phosphorus experiments, after the administration of xylose, 0.2 gm. glycine per kilo was injected into the marginal vein of the ear, and the amino nitrogen<sup>6</sup> of the blood determined before and two and one-half hours after injection. At the conclusion of each experiment, the animal was killed and an autopsy was performed. Urine, removed from the bladder, was tested for albumin and the sediment was examined; the kidneys and livers were saved for histologic examination.

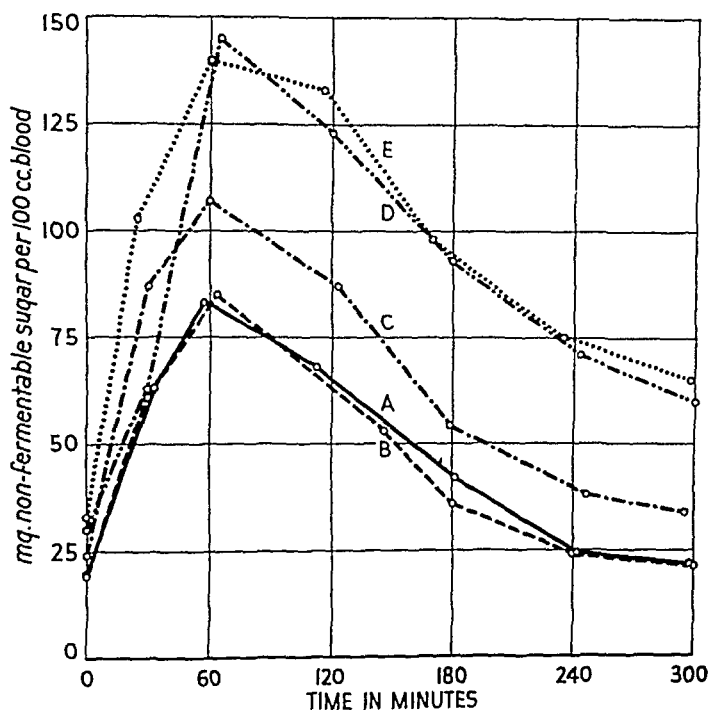


Fig. 1.—Effect of uranium intoxication in a rabbit as shown by the xylose tolerance. Normal tolerance curve A, before uranium injection; B, seventeen hours; C, two days; D, three days; E, four days after an injection of 0.5 mg. uranium acetate per kilo. Maximum effect occurs third or fourth day.

#### XYLOSE TOLERANCE

When xylose is administered orally to normal rabbits, the nonfermentable sugar of the blood rises to a maximum within one or two hours, after which it falls gradually until the initial or normal value is approached within five hours. According to Fishberg and Friedfeld<sup>1-3</sup> if the kidney function is impaired, the nonfermentable blood sugar may continue to rise even after five hours; and a correspondingly longer period of time, depending upon the amount of kidney damage, is required before the xylose is eliminated from the blood stream. It was thought that the course of uranium poisoning would manifest itself by progressively lower tolerance.

In Fig. 1 are shown the daily xylose tolerances of a rabbit after a single administration of 0.5 mg. uranium acetate per kilo. Seventeen hours after the uranium was given, the xylose curve checked with the normal. In this period the blood urea nitrogen had risen from 11.4 to 27.4 mg. The second day showed a considerable rise in the xylose curve, followed by a still greater rise on the third day. The fourth-day tolerance paralleled the third, so that the maximum effect of a single injection of uranium is reached about the third day after administration. This maximum effect varies in different animals, but appears between two and four days after injection.

In the present work, an effort was made to give the smallest dose of uranium which would produce sufficient kidney damage to be measured by the xylose

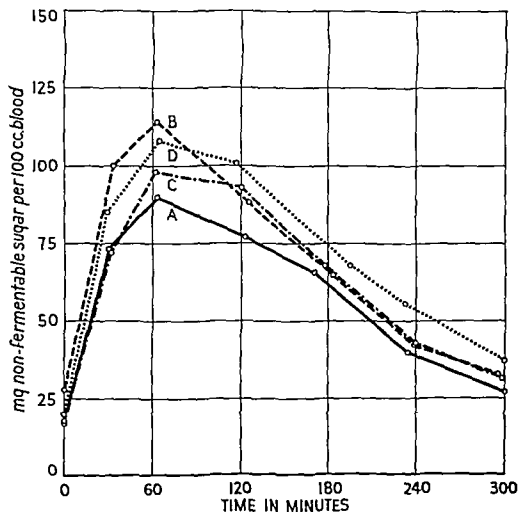


Fig. 2.—Acquired uranium resistance in a rabbit which had previously been intoxicated by small amounts of uranium. Xylose tolerance A, two months after 0.5, 0.5 and 1.0 mg. per kilo doses of uranium acetate; B, tolerance three days after additional 0.5 mg.; C, three days after 0.6 mg.; D, three days after 0.7 mg. uranium acetate per kilo, 11 days intervening between doses.

clearance, and then to increase this dose gradually. The susceptibility of rabbits to uranium varied greatly; some reacted to such small amounts as 0.2 or 0.3 mg. per kilo while with others these quantities seemed to have no effect. Five-tenths milligrams per kilo was found sufficient to produce the initial effect in most rabbits, but in three cases was enough to cause death. The rate of absorption of uranium also seemed to vary in different rabbits, and must be considered in determining the minimum toxic dosage. Age and sex must also be considered.

After repeated small doses, rabbits seem to develop a resistance to uranium intoxication. This is contrary to the work of Garnier and Marek<sup>7, 8</sup> who claim

that rabbits become accustomed to uranium only after increasing doses from 1 to such amounts as 40, 60, and 80 mg. per kilo with suitable time intervals intervening. They claim<sup>9</sup> that small doses and repetition of the same dose do not impart resistance, but that nitrogen excretion increases. According to Patrassi and Rogers,<sup>10</sup> rabbits treated with repeated small subcutaneous doses of uranium develop kidney lesions of a lesser degree on subsequent intravenous treatment than do control animals which have received intravenous injections of uranium without previous subcutaneous treatment. MacNider<sup>11</sup> has recently shown that dogs may develop resistance to uranium with repetition of 4 mg. per kilo doses. He found that in animals which survived this injection there was evidence of great disturbance in hepatic function as shown by the use of the phenoltetra-

TABLE I

XYLOSE TOLERANCE OF RABBITS BEFORE AND THREE DAYS AFTER INITIAL URANIUM INJECTION

RABBIT	URANIUM ACETATE	NONFERMENTABLE BLOOD SUGAR MG. PER 100 C.C. BLOOD							BLOOD UREA N
		BEFORE XYLOSE	MINUTES* AFTER INGESTION OF XYLOSE						
			30	60	120	180	240	300	
	mg. per kilo	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg. per 100 c.c.
113 ♀	0	24	65	84	72	52	35	26	13.0
	0.5	27	84	92	84	61	40	34	17.1
170 ♀	0	25	37	79	66	38	24	24	17.8
	0.5	29	75	84	74	61	43	33	16.8
188 ♀	0	18	46	85	59	35	25	18	13.0
	0.5	21	73	91	75	43	29	23	13.5
196 ♂	0	24	60	78	52	42	35	26	13.4
	0.5	24	61	71	56	53	46	37	18.3
223 ♀	0	26	36	88	87	56	45	31	14.8
	0.5	26	80	123	136	124	83	39	19.5
297 ♀	0	24	64	84	80	63	40	27	14.9
	0.5	28	56	83	98	71	52	32	18.0
304 ♀	0	23	66	73	54	43	31	20	11.0
	0.5	26	72	75	62	37	30	25	22.0
347 ♀	0	19	63	76	66	44	31	21	16.3
	0.5	25	67	97	89	67	44	28	27.1

\*Approximate.

chlorphthalein test. In animals surviving such a degree of liver injury there slowly occurs an improvement of hepatic function. Biopsy material obtained from the liver at this stage showed that the repair process to the epithelium had taken place through the formation of a different type of cell. When these animals were re-intoxicated with uranium, it was found that the liver had acquired resistance to this hepatotoxic substance. MacNider<sup>12, 13</sup> also found that the injection of 4 mg. per kilo uranium nitrate in dogs resulted in an epithelial injury to the kidney which was very largely localized to the proximal convoluted tubule epithelium. A certain number of dogs rendered acutely nephritic returned to complete normal function after a variable period allowed for renal repair. He found that restoration of such a function was accompanied by regeneration of epithelium of two different types. In one, the regenerated epithelium resembled histologically normal convoluted tubule cells while in the other, in

addition to these normal cells, there was a dominance of a low, flattened, apparently less specialized epithelium. Animals in which this latter type occurred were found to have developed a uranium resistance.

In this investigation relatively small amounts of uranium were used in comparison with those employed by MacNider, and Garnier and March. Uranium acetate in amounts from 0.2 to 10 mg. was employed. The usual dose was 0.5 mg., and the reactions of the rabbits to this amount varied greatly. The majority showed a considerable decrease in xyllose tolerance after initial

TABLE II

## XYLOSE TOLERANCE OF RABBITS AFTER REPEATED INJECTIONS OF URANIUM

DATE	RABBIT	NONFERMENTABLE BLOOD SUGAR MG PER 100 C C BLOOD							BLOOD UREA N MG PER 100 C C	URANIUM ACETATE INJECTED	
		DIFFER- ENCE XYLOSE	MINUTES* AFTER INJECTION OF XYLOSE							DATE	MG PER K
			30	60	120	180	240	300			
5/13	35 ♂	mg	mg	mg	mg	mg	mg	mg	22.9	5/20	0.5
5/20		21	63	59	87	56	76	29	31.4		
6/14		27	92	126	133	111	104	75	16.2	6/15	0.5
6/20		24	68	86	90	54	37	28	20.5	6/23	0.5
6/27		22	86	107	92	58	76	26	17.0	7/ 8	0.5
7/11		24	92	113	91	69	44	33	47.1		
7/29		27	92	110	103	72	57	43			
4/11	164 ♀	22	59	76	60	43	25	20	13.2	4/ 8	0.5
4/24		20	47	70	82	76	65	52	54.4	4/22	0.6
5/ 4		23	73	104	111	81	58	43	20.1	5/ 1	0.7
7/17		26	59	93	105	97	68	44	17.2	7/14	0.5
		20	60	80	86	69	47	37	20.2		
5/19	188 ♀	18	46	85	59	30	25	18	13.1	5/26	0.5
5/29		21	73	91	75	43	29	20	13.5	6/11	0.5
6/16		21	77	89	53	43	23	20	12.7	6/22	0.3
6/26		22	51	102	94	53		35	16.6	7/10	0.5
7/13		29	99	81	71		56	37	12.6		
3/30	326 ♂	18	59	77	56	70	25	18	11.5	4/10	0.5
4/13		20	90	113	118	105	89	63	26.8	4/24	0.6
4/28		22	89	107	114	100	73	50	19.3	5/ 5	0.7
5/ 8		21	68	91	111	80	58	40	20.9	7/15	0.5
7/19		24	77	66	75	57	37	27	26.2		
4/ 6	98 ♀†	18	73	90	77	66	40	27	16.4	4/11	0.5
4/14		28	100	114	88	64	42	2	20.0	4/22	0.6
4/25		17	72	98	93	68	43	30	19.1	5/ 6	0.7
5/ 9		20	85	108	101	68	56	7	27.0	7/15	0.5
7/18		30	82	109	82	68	48	0	22.3		

\*Approximate

†This rabbit had previously received 0.5 mg. uranium per kilo on 12/11, 0.25 mg. on 11/16, and 0.5 mg. on 1-23 and 2/25.

uranium intoxication, but in approximately a fourth of the animals this small amount either had no effect or there was a barely perceptible decrease in tolerance.

In Table I are listed the xyllose tolerances of rabbits before injection of uranium, and three days after the administration of a 0.5 mg. per kilo dose. Most of these rabbits show the minimum effect of this small dose. There is only a slightly lower tolerance as compared with the normal, and this lower tolerance is accompanied by a more pronounced elevation in blood urea nitrogen. Albumin varies greatly. In nephritic rats previously studied,<sup>14</sup> xyllose tolerance and urea retention did not show such close agreement.

Rabbits which showed a high degree of initial uranium intoxication were used in the experiments to determine whether or not a resistance could be developed to this toxic substance. In Table II are listed a few examples of the xylose tolerances of rabbits after repeated injections of uranium. These rabbits show marked kidney damage as a result of initial uranium poisoning. The tolerance is considerably lowered after the injection of a small amount of uranium, and there is a pronounced rise in the blood urea nitrogen. When the dose is repeated or slightly increased, after a suitable time interval has elapsed, the kidney seems to have acquired a resistance to uranium. The xylose tolerance again approaches the normal, and the blood urea nitrogen falls. Repeated doses of uranium appear to have little effect.

If, however, the time interval between doses is too short or too long this resistance is altered. It appears, therefore, that this acquired resistance is not a permanent one. Approximately a fourth of the animals treated failed to develop a definite uranium resistance. The lowered xylose tolerances of these rabbits may be due to individual variations of the animals and to a lack of a suitable time interval between uranium intoxications.

Rabbit 98 is an example of a group of rabbits which had been repeatedly intoxicated with small amounts of uranium over periods of one to four months. The xylose tolerances were then determined, and the animals were given increased amounts of uranium with little or no effect upon the xylose tolerance or blood urea nitrogen. In a few instances the tolerances and blood ureas rose considerably but later returned to normal after additional uranium treatment. There are individual variations and exceptions in the work with some rabbits which are difficult to explain.

When the rabbits in the above experiments were killed they showed pathologic evidence of nephritis and marked liver involvement. Histologic examination exhibited severe renal lesions, and in some rabbits, evidences of spontaneous nephritis. This brings up the question as to whether xylose clearance is a satisfactory test of renal function. If the above tolerances are considered in the light of the histologic reports, the answer would be in the negative. It would seem that in animals evidencing such marked nephritis the amount of xylose retained in the blood should have been considerably higher than that which was found. Fishberg and Friedfeld,<sup>1, 2</sup> working with rabbits with uranium-induced nephritis, report increased nonfermentable blood sugar values five hours after the administration of xylose. In the present investigation out of some 80 tolerance tests, the maximum nonfermentable blood sugar was obtained within two hours after ingestion of xylose, and at the end of five hours most values approached the normal. It is, therefore, apparent that the marked degree of nephritis reported here was not enough to cause any great retention of xylose, and it is evident that xylose tolerance cannot be considered a delicate indicator of renal function.

#### XYLOSE TOLERANCE IN PHOSPHORUS POISONING

In the clearance of various foreign substances from the blood stream, there is always the question as to whether or not more than one organ is involved. Fishberg and Friedfeld<sup>1, 2</sup> claim that liver injury does not affect the clearance

TABLE III  
XULOSE TOLERANCE OF RABBITS POISONED BY PHOSPHORUS

DATE	RABBIT	PHOSPHORUS MG PER KG		NONFERMENTABLE BLOOD SUGAR MG PER 100 C.C. BLOOD										BLOOD UREA N	GLYCINE INJECTION			
				MINUTES* AFTER INGESTION OF XYLOSE							BEFORE		BLOOD AMINO ACID N					
		MG	DATE	BEFORE XYLOSE							AFTER 2 1/2 HOURS							
				30	60	120	180	240	300	mg per 100 c.c.	mg per 100 c.c.	mg per 100 c.c.	mg per 100 c.c.					
2/15	9 ♂ †	0.2	2/15	mg	74	78	58	39	32	29	mg	25.8	88	11.2				
2/19		0.6	2/19	28	65	82	56	40	32	28	23.4	11.3	11.5					
2/23				22	85	88	59	43	28	25	22.5							
2/26	187 ♂ †	0.8	2/24	34	36	75	88	85	70	33.2	10.1	20.3						
1/15	196 ♂	0.6	2/16	27	82	58	42	33	25	20.3	9.2	23.0						
2/20		0.6	2/19	28	65	99	107	103	99	29.1								
3/ 3	385 ♀	1.0	2/26	31	58	66	71	80	71	23.4	12.2	20.0						
3/ 2	394 ♀	1.0	3/ 1	25	71	55	48	33	27	20.8	9.3	10.5						

\*Approximate

†One pole of left kidney ligated 12/1

‡One pole of left kidney ligated 12/1 Right nephrectomy 12/26

of xylose. In order to investigate this question rabbits were injected with various amounts of phosphorus in olive oil, and a few days later xylose tolerance and deaminizing experiments were run simultaneously. After the ingestion of xylose, 0.2 gm. glycine per kilo was injected into the marginal vein of the ear. The amino nitrogen of the blood was determined before injection, and after two and one-half hours. The results are given in Table III.

Some of the rabbits were strangely resistant to phosphorus poisoning. This may have been due to faulty absorption of the phosphorus. In these animals, destruction of the liver was not sufficient to interfere with its deaminizing function, and the xylose tolerance remained normal. However, in those animals in which there was any considerable rise in amino acid nitrogen of the blood, xylose retention was great. In three instances the maximum nonfermentable blood sugar maintained a practically constant high level for five hours after xylose administration. It would therefore seem that where liver injury is sufficient to affect its deaminizing function, this injury may be manifested by giving very high xylose tolerances. A right nephrectomy and a ligation of one pole of the left kidney had previously been performed on one of the animals (Rabbit 187) used in this experiment. In a control animal similarly treated, the xylose tolerance was normal.

#### GALACTOSE TOLERANCE

A considerable amount of work was done with galactose tolerance, but space limitation prevents a detailed report and the inclusion of tables. It was found that galactose tolerance in normal rabbits was practically the same as xylose tolerance. What was said about dosage and resistance to uranium poisoning in the preceding work with xylose is also true of galactose. The majority of animals responded to relatively small amounts of uranium as evidenced by a decreased galactose tolerance and an increase in the blood urea nitrogen. The small amounts of uranium given were probably not sufficient to cause liver injury and one may conclude, therefore, that the kidney injury by uranium poisoning caused retention of the galactose. This would seem to indicate that retention of galactose is not solely an indication of damaged liver function.

#### SUMMARY

After injection of very small amounts of uranium, xylose tolerance is decreased. After repeated injection of these small amounts, rabbits seem to develop a uranium resistance. Considering the extent of kidney damage produced by uranium, the tolerance of xylose cannot be considered a delicate indicator of renal function. When the liver is severely injured by phosphorus poisoning, xylose tolerance may be greatly decreased.

Galactose tolerance in normal rabbits is practically the same as xylose tolerance. Kidney damage produced by uranium poisoning may lower galactose tolerance.

The author is indebted to Dr. E. M. Medlar for the histologic examinations. He wishes to express his appreciation to Dr. N. R. Blatherwick for his many helpful suggestions, and to Phoebe Bradshaw, Anna Post, and Owen Cullimore for valuable assistance in the work. Pure xylose was generously furnished by the United States Bureau of Standards.

## REFERENCES

- 1 Fishberg, E. H., and Friedfeld, L. The Excretion of Xylose as an Index of Damaged Renal Function, *J Clin Investigation* 11 501, 1932
- 2 Fishberg, E. H., and Friedfeld, L. Excretion of Xylose as a Measure of Renal Function in Children, *Am J Dis Child* 45 271, 1933
- 3 Fishberg, E. H., and Friedfeld, L. Ausscheidung von Xylose als Massstab der Nierenfunktion, *Klin Wchnschr* 12 218, 1933
- 4 Folin, O., and Malmros, H. An Improved Form of Folin's Micro Method for Blood Sugar Determinations, *J Biol Chem* 83 11, 1929
- 5 Van Slyke, D. D. Determination of Urea by Gasometric Measurement of the Carbon Dioxide Formed by the Action of Urease, *J Biol Chem* 73 695, 1927
- 6 Folin, O. A System of Blood Analysis. A New Colorimetric Method for the Determination of the Amino Acid Nitrogen in Blood, *J Biol Chem* 51 377, 1922
- 7 Garnier, M., and Marek, J. Les limites de l'accoutumance au nitrate d'urane en injection sous cutanee chez le lapin, *Compt rend Soc de biol* 103 1077, 1930
- 8 Garnier, M., and Marek, J. Effets des doses croissantes de nitrate d'urane en injection sous cutanee chez le lapin, *Compt rend Soc de biol* 108 611, 1931
- 9 Garnier, M., and Marek, J. Des effets de la repetition d'une meme dose de nitrate d'urane en injection sous cutanee chez le lapin, *Compt rend Soc de biol* 107 99, 1931
- 10 Patrassi, G., and Rogers, N. W. Alterazioni renali e manifestazioni immunitarie nell'intossicazione sperimentale da uranio, *Sperimentale Firenze* 85 259, 1931
- 11 MacNider, W. de B. Acquired Resistance of Liver Cells to the Toxic Action of Uranium Nitrate, *Proc Soc Exper Biol & Med* 32 791 1935
- 12 MacNider, W. de B. The Development of the Chronic Nephritis Induced in the Dog by Uranium Nitrate. A Functional and Pathological Study With Observations on the Formation of Urine by the Altered Kidneys, *J Exper Med* 49 387, 1929
- 13 MacNider, W. de B. The Functional and Pathological Response of the Kidney in Dogs Subjected to a Second Subcutaneous Injection of Uranium Nitrate, *J Exper Med* 49 411, 1929
- 14 Tarrson, H. W. Comparison of the Xylose Tolerance With Blood Urea in Nephritic Rats, *J LAB & Clin Med* 21 1010, 1936

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## AGRANULOCYTOSIS IN THE NEGRO\*

### CASE REPORT WITH ETIOLOGY AND COMMENT

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**A**GRANULOCYTOSIS is a rare disease in negroes. In 1930 Tallv and Griffith<sup>1</sup> reported one other such case in which the neutropenia occurred following the administration of asphenamine. Grady Hospital has 225 beds for negro patients, and in eight years only one individual with agranulocytosis has been admitted to its wards. In no instance has the disease been observed in the outpatient clinic where more than 20,000 negroes are treated each year. The occurrence of this malady so rarely in negroes necessarily calls for etiologic speculation, and it is important and of great interest to know, if possible, the reason for its rarity in the negro race.

The etiology of agranulocytosis has not been entirely disclosed. Kiacke and Parker<sup>2</sup> have presented clinical and experimental evidence that benzamine drugs (chiefly amidopyrine) are the predisposing etiologic agents, and their observations and claims have been confirmed by other observers in America and European countries.

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As regards the infrequency of the disease, Kracke<sup>3</sup> feels that the negroes do not have neutropenia, because they do not use drugs such as amidopyrine. There seems to be sound reason for this opinion. We who are southern physicians are well aware of the negro's aptitude and ability to undergo severe pain. The negro may be extremely emotional at times, but he wears his pain and suffering with grace and humility. His therapeutic agents are limited by necessity to simple remedies such as liniments, castor oil, epsom salts, hot pepper, turpentine, roots and herbs. When a headache occurs he applies liniment, uses a charm, and wears off the pain; or he may apply a tight bandage about his skull to "squeeze out the misery." Not infrequently we observe negroes in our hospital who undoubtedly have angina pectoris, yet it is difficult to obtain a description of the anginal manifestations as we have them described by white people. The negro will simply refer to "misery in my chest" and let it go at that. We have long since learned that such complaints in negroes may mean many things. Negro women, in contrast with white women, seldom complain of menstrual pain; however, this particular problem would open a wide field for consideration which is unnecessary in this report.

The statements herein so far made are intended to indicate that inasmuch as the negro bears his pain more stoically than many of the white people, he therefore finds it unnecessary to buy "pain killers." It is not amiss also to refer to the economic side of the matter. Certainly the poorer classes cannot, or do not, buy expensive analgesics, and we also recall how few cases of agranulocytosis we have seen in the poorer white people. Those of this class who are suburban and who come to the white wards of Grady Hospital likewise seldom have agranulocytosis.

If the negroes do not have granulopenia because they do not use benzamine drugs, then Kracke's etiologic claim is herein strongly supported. If they have not agranulocytosis because of other reasons, then the field of etiologic investigation increases in interest and widens the research scope. It is in order, therefore, that all such patients be studied thoroughly and reports made until the question of etiology is solved.

#### CASE REPORT

T. F. (No. 72339.) Female, aged twenty-four years. Admitted to the hospital on June 3, 1935, and died June 13, 1935, following an illness of forty-five days' duration.

*The chief complaints* were of fever and headache, which had existed for five weeks prior to admission to the hospital. She had not been entirely confined to her bed until one week before admission, at which time she was given at the clinic an injection of milk, the resulting reaction forcing her to bed. A day later she developed a sore throat, a nasal discharge, and slight deafness. The throat soreness became severe and she could not drink fluids. She had pain in her face and neck, and about both sides of her cheeks.

*Past History.*—The patient had always suffered pain during menstruation, and in recent months the pains were quite severe. She has also had a vaginal discharge for some time past which had recently increased. She had no children nor had she any miscarriages. No other illness of importance was described except a mild chronic gastric distress, bleeding hemorrhoids, and constipation. She had lost ten pounds in weight in the last thirty days.

*Physical Findings.*—She was a well-developed and well-nourished female, whose blood pressure was 112 systolic and 74 diastolic. She had fever of 104° F. and a pulse rate of 100. Her pupils were equal. The sclera were slight yellow. Her neck was not rigid. There were dental caries and swollen inflamed gums. A diffuse pharyngeal inflammation of a brownish

red color, and a yellow postnasal discharge were present. The tonsils were apparently uninvolvement. All other findings were normal except lower abdominal tenderness on pressure with a slight rigidity of the abdominal muscles, enlarged liver, cervical discharge, internal and external hemorrhoids, and probable tubal ovarian masses as indicated in pelvic examination.

*Course in Hospital*—The patient lay quietly in bed. She became rapidly dehydrated. When moved about she complained of pain and weakness. Her throat lesions continued. A definite jaundice became marked. She remained conscious, but appeared to be quite seriously ill. Her fever continued and averaged  $103.5^{\circ}\text{F}$ . On the sixth, seventh, ninth, and tenth days she had chills.

On June 4, her first blood study revealed 3,100 leucocytes, 3,200,000 red cells, hemoglobin 60 per cent, of the leucocytes 64 per cent were lymphocytes. Twenty-four hours later there were 1,150 leucocytes per c mm with 72 per cent lymphocytes. On June 6, there were only 850 leucocytes per c mm of blood and only lymphocytes could be found. Later, on the same date, 400 white cells were present and on the twelfth day of June the total white blood count was 150 cells per c mm. No granulocytes were found. During the interim the red cells had decreased to 2,100,000. The icterus index was 32 and the fragility test normal. Wassermann negative, Widal negative, nonprotein nitrogen 33 mg, malarial parasites not found, coagulation time four minutes, bleeding time 15 minutes, platelets 128,000 per c mm of blood. Four blood cultures remained free of bacteria. On June 13, 1935, she became extremely weak and died.

*Treatment*—The patient was given liver extract on June 11, 1935, though it was realized that little good could be accomplished. She was also given a transfusion, which was of no avail, and fluids, glucose, tepid sponges, douches, sodium perborate gargles and morphine. Unfortunately, when first admitted, she was given pyramidon, 10 gr (only two doses). This drug was omitted two days later on account of the low leucocyte count. On the tenth day, sterile turpentine was administered, intramuscularly, but all treatment failed to produce beneficial response.

*Discussion of Case*—This patient presented many of the cardinal features of primary granulopenia: weakness, sore throat, pain over face and absence of circulating neutrophils with practically total absence of all cells at death. After the diagnosis was established, the etiologic agent was sought. The patient denied taking pyramidon or amidopyrine previous to her illness nor had she taken any drug of an obscure type. The pelvic inflammatory disease did not impress her physicians as being seriously significant. There was a story related by her that does seem significant and that relates to her deafness, which had recently increased in severity. She admitted taking quinine to control her fever at the beginning of her illness, and quinine is a drug containing the benzamine nucleus. I am inclined to feel that she must have consumed considerable quinine to have caused progressive diminished hearing. With these facts at hand this patient may be considered a person with granulopenia probably resulting from quinine intoxication, possibly allergic, until further investigation discloses other etiologic agents. However, the possible connection is obscured by the administration of 10 gr of pyramidon on admission, since it has been shown that this amount of this drug is capable of producing profound depression of the leucocyte count.<sup>4,6</sup>

Only one instance of agranulocytosis resulting from quinine has been reported.<sup>7</sup> In this regard it is hoped that someone will undertake to evaluate quinine as a possible cause of the disease. As a rule patients with malaria have leucopenic blood, and occasionally malaria, a curable preventable disease, causes death. I recall that in fatal cases, presumably nonrefractory to therapeutic quinine, those patients have marked leucopenia, at times terminal hemoi-

rhages, and a mental confusion such as occurs often in the typical agranulocytic patient. The question arises, therefore, concerning the dangerous effects of quinine on the white blood cells of those patients, and the matter should be investigated, for it has long been known that quinine may produce allergic reactions.

#### REFERENCES

1. Tally, J. C., and Griffith, G. C.: Discussion of Six Cases of Agranulocytosis, *M. Clin. N. Amer.* 13: 1079, 1930.
2. Kracke, R. R., and Parker, F. P.: The Etiology of Granulopenia. With Particular Reference to the Drugs Benzene Ring, *J. LAB. & CLIN. MED.* 19: 799, 1934.
3. Kracke, R. R.: Personal conversation with author. November, 1935.
4. Sturgis, C. C.: *Tr. A. Am. Physicians* 49: 328, 1934.
5. Benjamin, J. E., and Biederman, J. B.: Agranulocytic Leukopenia. Report of a Case Successfully Treated With X-rays. Effect of Amidopyrine, *J. A. M. A.* 103: 161, 1934.
6. Zinberg, I. S., Katzenstein, Lawrence, and Wice, L. E.: Neutropenia, *J. A. M. A.* 102: 2098, 1934.
7. Groen, J., and Gelderman, C. J.: Agranulocytose (Maligne Neutropenie) door Due Geneesmiddelen, *Nederl. tijdschr. v. geneesk.* 78: 3444, 1934.

### THE RATE OF DISINTEGRATION OF PLATELETS\*

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THUS far studies on blood platelets have been limited largely to numerical determinations, at times supplemented by an examination of their morphologic features as revealed by dry, stained blood films. General observations on the disintegration of platelets have been made by a number of investigators, but the actual rate at which this disintegration proceeds has until recently received little attention despite the important rôle the fragility of platelets is considered to play in conditions such as hemophilia and hereditary hemorrhagic thromboasthenia. In this investigation simultaneous observations were made on the rate of disintegration, total and differential counts of the thrombocytes, and an attempt was made to analyze the results from the standpoint of their clinical significance.

A satisfactory method for determining the rate of disintegration of platelets should include simultaneous observations on the total and differential counts, thus correlating the qualitative properties, quantitative aspects, and morphologic features of the thrombocytes. The various methods that have been employed for studying the fragility of platelets can be generally divided into indirect and direct.

#### INDIRECT METHOD

In this method, advocated by Irish,<sup>1</sup> a suspension of platelets in hypotonic salt solution is brought in contact with platelet-free plasma or a solution of fibrinogen, thus completing the clotting system. The fragility is expressed as

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the concentration of sodium chloride just sufficiently hypotonic to cause disintegration of enough platelets to facilitate rapid clotting. The platelets are first washed free from plasma and then suspended in physiologic saline, a procedure involving unavoidable loss and destruction of some of the thrombocytes. The method, moreover, does not permit simultaneous total and differential platelet counts.

#### DIRECT METHODS

These methods involve the use of the microscope. The disintegration of platelets can be observed either by following the changes taking place in the thrombocytes found in one or more microscopic fields or by determining at regular time intervals their quantitative variations. The various platelet suspension media that have been employed for this purpose are (1) fresh undiluted blood, (2) physiologic solutions, (3) anticoagulants, and (4) anticoagulant preservatives.

1 *Fresh Undiluted Blood*—The earliest observations on the disintegration of platelets were made by employing platelets suspended in fresh undiluted blood. Later Mayer<sup>2</sup> and Stubel<sup>3</sup> and more recently Tait and Burke<sup>4</sup> and Ferguson<sup>5</sup> supplemented these studies by the use of the dark field microscope. In undiluted blood, however, the thrombocytes break up so rapidly that it is impossible to correlate by accurate simultaneous observations the fragility with the total and differential counts.

2 *Physiologic Solutions*—Platelets suspended in diluted blood disintegrate more slowly than when suspended in undiluted blood. Attempts have, therefore, been made to carry out in preparations of diluted blood simultaneous observations on the fragility and total differential counts of the platelets. Flossner,<sup>6</sup> Hofmann,<sup>7</sup> Boshamer<sup>8</sup> and Horwitz<sup>9, 11</sup> employed Tyrode's<sup>12</sup> solution as diluent. This diluent is a good artificial nourishing fluid, but a poor platelet preservative. In this solution the platelets begin to disintegrate after about twenty to thirty minutes, a time interval too brief for accurate determination of the total and differential platelet counts. Birch<sup>13</sup> and Kugelmass<sup>14</sup> used hypotonic salt solution as diluent, making no attempt to correlate the fragility of the platelets with the total and differential counts. I have found both Tyrode's solution and physiologic saline extremely unsatisfactory. The formed cellular blood elements are poorly preserved in these diluents and become considerably distorted after a short time. The results obtained by employing these solutions appear to be entirely untrustworthy.

3 *Anticoagulants*—Aynaud,<sup>15, 16</sup> Baar and Székely,<sup>1, 18</sup> Baar<sup>19</sup> and Kiguchi and Mizuta<sup>20</sup> employed sodium citrate as anticoagulant. Aynaud used also sodium oxalate. These anticoagulants, however, do not preserve platelets and therefore possess no advantages over diluents such as physiologic sodium chloride or Tyrode's solution. Preiss<sup>21</sup> who used a mixture of Tyrode's solution and heparin failed to observe any significant changes in the total platelet count at the end of one hour, the differential formula, however, at the end of the same period of observation revealed a decrease in the smaller platelets and an increase in the larger forms.

4. *Anticoagulant Preservatives.*—The anticoagulants employed here possess the property of preserving platelets. Aynaud<sup>15, 16, 22</sup> studied the disintegration of platelets by using as diluents solutions of sodium arsenite, urea and sodium metaphosphate. More careful observations on the rate of disintegration of platelets were made by Baar and Székely<sup>17, 18</sup> and König.<sup>23</sup> The former investigators used a 10 per cent solution of urea originally recommended by Kristenson<sup>24</sup> for platelet enumeration, the latter a 14 per cent solution of magnesium sulphate. These diluents are definitely hypertonic and readily break up platelets. Urea, moreover, hemolyzes red cells. The hemolysis of red cells, by increasing the osmotic pressure, produces further alterations in the platelets. Neither the magnesium sulphate nor the urea solutions, therefore, are suitable diluents for making accurate observations on the fragility, total and differential counts of the platelets.

#### METHOD

*The Diluent.*—In this investigation a solution of sodium metaphosphate was employed for determining the rate of disintegration, total and differential counts of the platelets. It has the following composition:

	GM. OR C.C.
Sodium metaphosphate (Howe and French)	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

The procedures can be carried out simultaneously since this diluent, originally recommended by Pratt<sup>25, 26</sup> for platelet enumeration, preserves the thrombocytes for a sufficiently long period of time, at least two hours. In fact, in a 5 per cent solution of sodium metaphosphate the total number of thrombocytes has been observed by Pratt<sup>27</sup> to remain at times unchanged for days.

Some investigators advise against the use of anticoagulant preservatives as blood diluents for determining the fragility of platelets, since these at times produce considerable alterations in the thrombocytes. As a matter of fact some changes in the platelets take place immediately upon their extravascular escape due to a number of unavoidable factors: contact with tissue juices and disintegration products of cells; changes in temperature, osmotic relations and hydrogen ion concentration; disturbances of normal blood gas equilibria due to the escape of volatile substances. Even physiologic sodium chloride solution has been shown by Morawitz,<sup>28</sup> Schittenhelm and Bodong<sup>29</sup> and Abderhalden and Deetjen<sup>30</sup> to damage platelets. Extravascularly the platelets are always abnormal regardless of the medium in which they are suspended. The abnormality in the thrombocytes may reach a considerable degree in diluents like magnesium sulphate or urea previously discussed. Sodium metaphosphate, however, as an anticoagulant preservative, has been shown by Morawitz<sup>28</sup> to possess the unusual characteristic of preserving the platelets without producing any changes in their ferments or precursors of ferments, so that platelets suspended in this diluent retain their properties of coagulating blood. Furthermore, in the concentration employed here the sodium metaphosphate solution does not produce any distortion of the cellular blood elements which appear perfectly normal for many hours. Aynaud<sup>22</sup> and Bürker<sup>31</sup> made similar observations. The sodium

metaphosphate solution, therefore, as a platelet preservative is to be preferred to the previously described diluents, as it produces minimum alterations in the thrombocytes

*The Total Platelet Count*—The method employed for the enumeration of the platelets is described by me in detail elsewhere<sup>32</sup> It may be outlined briefly as follows

The palmar surface of the finger tip is punctured with an automatic lancet after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether The first drop or two of blood is discarded A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin, and the hand quickly turned over so that the palmar surface is directed downward After a sufficiently large drop of blood has escaped into the drop of diluent, the mixture is applied to the surface of a small quantity (three to four drops) of diluting fluid contained in a paraffin cup The mixture in the cup is stirred and then transferred by means of a paraffin coated applicator to a glass slide, usually three preparations can be obtained as the quantity of fluid in the cup yields three large drops A cover slip is placed over each drop and after the preparations have been allowed to stand for ten to fifteen minutes, a relative thrombocyte erythrocyte count is made, using the oil immersion lens Sealing the edges of the preparations with liquid petrolatum will prevent the production of air currents An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined With this method the average number of platelets per cubic millimeter in normal adults is about 500,000

*The Differential Platelet Count*—A complete discussion of the method employed for determining the size of the platelets in wet preparations and the clinical significance of the deviations from the normal will be found elsewhere<sup>33</sup> The size of the platelets is estimated in a manner similar to the determination of the average diameter of red cells The procedure is carried out simultaneously with the enumeration of the thrombocytes The platelets, as observed in wet preparations, can be divided according to size into four groups Group I, consisting of platelets whose size is one quarter the diameter of a red cell, or about 1.8 microns, Group II, consisting of platelets one third the diameter of a red cell, or about 2.5 microns, Group III, consisting of platelets one half the diameter of a red cell or greater, or about 3.6 microns, and Group IV, consisting of irregularly shaped platelets The differential formulas from 45 normal adults yielded the following averages Group I, 18.6 per cent, Group II, 63.3 per cent, Group III, 17.4 per cent, Group IV, 0.7 per cent There is considerable evidence indicating that the smaller platelets, those belonging to Group I, are the younger forms<sup>33</sup>

*The Rate of Disintegration of Platelets*—The rate of disintegration of platelets in the sodium metaphosphate solution can be studied by mixing a drop of freshly drawn blood with several drops of diluent contained in a paraffin cup and then determining the variations in the platelet count at regular time intervals The results obtained by using the blood from a normal young adult are shown in Chart 1 It is apparent from this chart that, during the first two hours, the variations in the number of platelets are slight This is followed during the succeeding five to six hours by a considerable drop in their numbers After this point the numerical variations are of small magnitude, owing to the

slow disintegration of the remaining platelets. Based on these observations a method was devised for determining the rate of disintegration of thrombocytes. The technic is as follows:

Capillary blood is mixed with the preserving fluid by the method previously described for enumerating platelets. Two separate paraffin cups, each containing several drops of diluted blood, are prepared. Initial total and differential platelet counts are obtained by using the solution contained in one of the cups. The remaining cup is placed in an ice box and kept there at a temperature of approximately  $10^{\circ}$  C. At the end of about eight hours, total and differential platelet counts are obtained by using the solution contained in the second cup.

The rate of disintegration of the platelets as revealed by the decrease in the total platelet count at the end of eight hours shows considerable variations in normal individuals. If the total number of platelets lost at the end of the

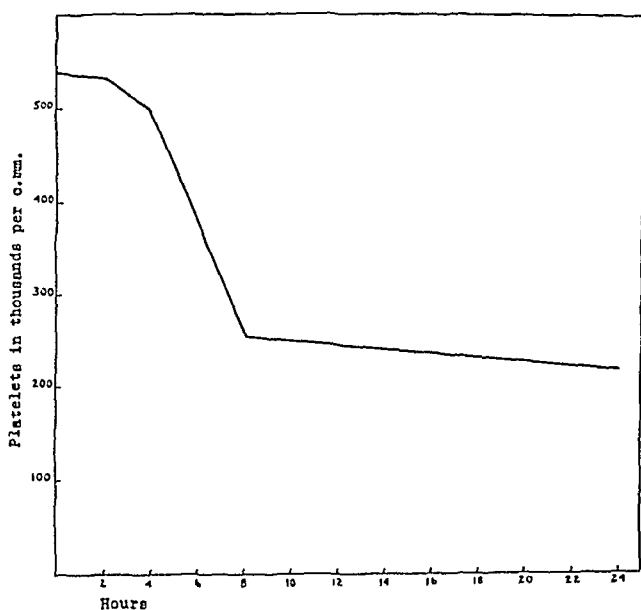


Chart 1.—Normal rate of disintegration of platelets.

observation period is divided by the initial count and the quotient multiplied by 100, the disintegration index (D. I.) in percentage is obtained. For example, if the initial count is 500,000 platelets per c.mm. and the eight-hour count 300,000, the disintegration index is 40; in other words, 40 per cent of the platelets have become completely disintegrated. In a group of 26 normal individuals 85 per cent revealed a disintegration index varying from 11 to 60, and 50 per cent an index ranging from 21 to 40.

The differential platelet formula undergoes very definite changes during the course of disintegration of the platelets. This is illustrated by Chart 2 and Table I.

Obviously the significant changes in the differential platelet formula at the end of eight hours are due to a relative and absolute decrease in the number of the larger platelets, i.e., those belonging to Groups II and III, and to a relative,

and usually also an absolute increase in the smaller forms, those belonging to Group I. The platelets of Group IV usually play a rather insignificant rôle. As a result of the disintegration of the platelets, there is a gradual shift of the differential formula to the left. The largest platelets, those belonging to Group III, presumably disintegrate into two or more fragments which then become members of either Groups I or II. The platelets of Group II disintegrate in turn to become units of Group I.

Apparently the larger, more mature platelets are more fragile and disintegrate more readily than the smaller platelets. Further support for this view is offered by the morphologic and quantitative changes of the thrombocytes following the subcutaneous injection of 1 c.c. of adrenalin chloride (1:1,000), as shown in Table II.

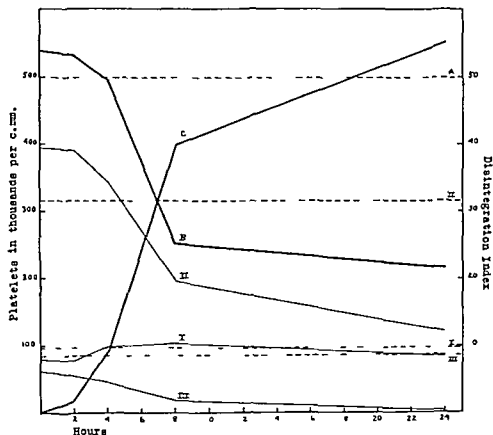


Chart 2—Behavior of the disintegration index and the differential formula during the normal course of the disintegration of platelets. Dotted line A represents the normal platelet level; dotted lines B, C, and D the normal absolute levels of the corresponding groups. Continuous line E represents the behavior of the total platelet count of a normal subject; continuous lines F, G, H, and I the absolute counts of the corresponding groups. Continuous line J represents the behavior of the disintegration index.

The characteristic feature of adrenalin thrombocytosis is the appearance of increased numbers of large platelets which must come from inactive blood reservoirs where normally hemopoiesis does not occur. They must consequently represent platelets that have remained inactive in the blood depots where many of them have matured, and were forced into the general circulation following the injection of adrenalin; they are, therefore, older or senile platelets. Associated with the shift of the differential formula to the right is an increase in the disintegration index. This increase in the fragility of the platelets as associated with a simultaneous increase in the larger types of thrombocytes would indicate that the larger forms disintegrate more readily than the smaller platelets. In a patient with bronchial asthma there was a slight decrease in the

disintegration index following the injection of adrenalin, associated, however, with an increase in the platelets of Groups I and III and a decrease in those of Group II. It would, therefore, seem that the sequence of disintegration of platelets is as represented in Fig. 1.

TABLE I  
CHANGES IN THE TOTAL AND DIFFERENTIAL COUNTS RESULTING FROM  
THE DISINTEGRATION OF PLATELETS

PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
		GROUP I		GROUP II		GROUP III		GROUP IV			
		0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
418,000	310,000	26.9	41.0	62.3	50.0	10.8	9.0	0.0	0.0	25.8	Normal
519,000	342,000	25.0	50.0	67.0	48.0	6.0	2.0	2.0	0.0	34.1	Normal
500,000	316,000	21.0	45.0	67.0	49.0	12.0	6.0	0.0	0.0	36.6	Normal
469,000	204,000	14.0	39.2	65.0	52.0	21.0	8.8	0.0	0.0	55.1	Normal
527,000	384,000	9.1	36.0	67.4	57.0	21.0	7.0	0.5	0.0	27.1	Normal
900,000	993,000	42.6	65.9	41.7	27.4	6.1	2.2	9.6	4.5	+10.3	Polycythemia vera (Case 9, Table IV)

TABLE II  
CHANGES IN THE DISINTEGRATION INDEX, TOTAL AND DIFFERENTIAL PLATELET COUNTS  
FOLLOWING THE INJECTION OF ADRENALIN

	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
			GROUP I		GROUP II		GROUP III		GROUP IV			
	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
Before adrenalin injection	445,000	268,000	26.0	----	62.5	----	11.5	----	----	----	40.0	Normal
After adrenalin injection	604,000	106,000	21.9	----	70.1	----	8.0	----	----	----	82.4	
Before adrenalin injection	29,000	29,000	16.7	----	33.3	----	50.0	----	----	----	0.0	Chronic thrombo- cytopenic purpura
After adrenalin injection	50,000	31,000	0.0	----	60.0	----	40.0	----	----	----	38.0	
Before adrenalin injection	252,000	217,000	32.8	----	50.4	----	16.8	----	----	----	13.8	Aleucemic myelosis
After adrenalin injection	518,000	331,000	24.6	----	59.1	----	16.2	----	----	----	36.1	
Before adrenalin injection	252,000	152,000	25.0	----	55.0	----	20.0	----	----	----	39.6	Bronchial asthma (during seizure)
After adrenalin injection	407,000	253,000	27.8	----	46.7	----	25.5	----	----	----	37.8	
Before adrenalin injection	402,000	237,000	61.8	----	35.2	----	3.0	----	----	----	41.0	Catarrhal jaundice
After adrenalin injection	575,000	284,000	28.5	----	68.0	----	3.5	----	----	----	50.6	

Under abnormal conditions the number of small platelets at the end of the observation period may be very high. This may be due to a very rapid disintegration of the larger platelets or to a slow rate of disintegration of the smaller forms, or to a combination of both factors. Under these circumstances the disintegration index may be 0 or even greater, i.e., the final count may be equal to or greater than the initial count (a disintegration index greater than 0 is preceded by a + sign). In general, a disintegration index of less than 10 or greater

than 0 indicates abnormal stability of the platelets. However, a disintegration index of 0 based on numerical calculations implies that none of the platelets in the specimen of blood examined have disintegrated within the period of observation. This conception is incorrect unless the associated differential formula remains unchanged or nearly so, revealing none or only slight changes at the end of the observation period. This is illustrated by a patient with polycythemia vera (Table I) in whom the final count was greater than the initial count. Despite this increase in the total eight hour count, the differential formula shows that 51 per cent of the platelets of Group IV, 39 per cent of Group III, and 143 per cent of Group II, or an absolute total of about 178,000 platelets per cmm have become disintegrated in the course of eight hours. A consideration of the changes in the differential platelet formula is, therefore, at times important in evaluating the results obtained following the disintegration of the thrombocytes.

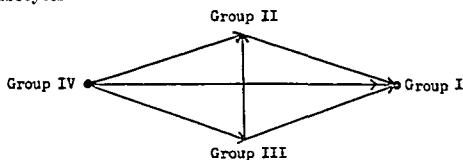


Fig 1—Sequence of disintegration of platelets

#### THE RATE OF DISINTEGRATION OF PLATELETS IN DISEASE

*Anemia*—In the chronic anemias, secondary and primary, and in the anemias due to acute blood loss, the disintegration index is normal (Table III), the platelets in these conditions disintegrate fairly rapidly. As the anemia improves with iron or liver therapy, the disintegration index tends to decrease. There is a simultaneous rise in the total platelet count to the normal level and a change in the differential formula characterized by a shift to the left due to an increase in the number of the smaller thrombocytes. In these conditions the long standing thrombopenia, consisting of platelets of moderate fragility, is followed by a normal thrombocyte count consisting of platelets relatively more resistant to disintegration. The disintegration index eventually becomes normal, as illustrated by Case 5 who has been under liver treatment for a long time.

*Polycythemia Vera*—Untreated cases of polycythemia vera usually yield normal disintegration indices (Cases 6 and 7, Table IV). Patients in Cases 8 and 9, treated with phenylhydrazine, yielded disintegration indices greater than 0 i e, the final total platelet counts were greater than the initial counts. That some of the platelets have disintegrated is apparent from the fact that in Case 8 at least 7 per cent of the platelets of Group III and 9 per cent of Group I, or an absolute total of about 70,000 platelets per cmm, have disappeared at the end of eight hours. Similarly in Case 9, 51 per cent of the platelets of Group IV, 39 per cent of the platelets of Group III and 143 per cent of Group II, or a total of about 178,000 platelets per cmm have disintegrated. However, even when considered from this aspect, the rate of disintegration of the platelets in these polycythemias treated with phenylhydrazine is rather slow.

This is in agreement with the observations of Morawitz and Pratt<sup>34</sup> and Itami and Pratt<sup>35</sup> who noted an increased resistance of the red cells in experimental phenylhydrazine anemias.

TABLE III  
DISINTEGRATION INDEX IN ANEMIAS

CASE	SEX	AGE	PLATELETS PER C.M.M.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.				
1	M	33	250,000	136,000	29.2	48.0	39.8	48.0	27.7	4.0	3.3	0	45.6	Chronic bleeding peptic ulcer; R.B.C. 2.29 mil. One month later; R.B.C. 5.42 mil.
			488,000	440,000	36.4	32.0	50.0	56.0	12.5	12.0	1.1	0	9.8	
2	M	55	674,000	490,000	60.0	42.0	35.0	58.0	5.0	0.0	0.0	0	27.3	Acute bleeding peptic ulcer.
3	M	56	293,000	193,000	14.7	62.0	55.4	34.0	27.4	4.0	2.5	0	34.1	Primary hypochromic anemia. After a month's treatment with iron.
			451,000	332,000	22.7	34.0	56.4	60.0	20.0	6.0	0.9	0	26.3	
4	M	68	398,000	213,000	33.3	32.0	42.0	60.0	24.7	8.0	0.0	0	46.5	Pernicious anemia. After 6 weeks of liver treatment.
			508,000	364,000	32.2	55.0	47.1	38.0	20.7	7.0	0.0	0	28.3	
5	M	37	421,000	226,000	19.4	41.0	60.5	46.0	20.1	13.0	0.0	0	46.3	Pernicious anemia under liver treatment for a long time.

TABLE IV  
DISINTEGRATION INDEX IN POLYCYTHEMIA VERA

CASE	SEX	AGE	PLATELETS PER C.M.M.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	REMARKS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
6	F	50	581,000	377,000	25.8	34.0	41.0	50.0	29.0	16.0	4.2	0	35.1	Untreated
7	M	46	714,000	492,000	58.7	63.0	37.3	36.0	1.6	1.0	2.3	0	31.1	Untreated
8	M	60	462,000	470,000	25.0	16.0	40.0	56.0	35.0	28.0	0.0	0	+ 1.7	Under phenylhydrazine treatment.
9	F	50	900,000	993,000	42.6	65.9	41.7	27.4	6.1	2.2	9.6	4.5	+10.3	Under phenylhydrazine treatment.

Phenylhydrazine, when administered to patients with polycythemia vera, is well known to cause at times enormous increases in the number of circulating platelets. According to Jürgens and Bach<sup>36</sup> this phenylhydrazine thrombo-

cytosis constitutes an important predisposing factor in the causation of spontaneous thrombosis and embolism in these patients. However, the rather slow rate of disintegration of platelets during this form of therapy tends to counteract the thrombophilia. It is perhaps for this reason that spontaneous thrombosis in polycythemics treated with phenylhydrazine is not seen more frequently.

*Essential Thrombocytopenic Purpura*—The disintegration index was 0 in two patients presenting marked thrombopenia (Cases 11 and 12, Table V). Moderate thrombopenia, however, may show a normal rate of disintegration. This is shown by Case 10, Table V, the patient had an extremely abnormal

TABLE V  
DISINTEGRATION INDEX IN CHRONIC THROMBOCYTOPENIC PURPURA

CASE	SEX	AGE	PLATELETS PER C MM		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D I	REMARKS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR		
*10	M	25	127,000 406,000	88,000 322,000	10 55	20 20	150 226	60 180	840 719	920 800	0 0	0 0	30.7 20.7	After three blood trans fusions
11	F	27	55,000	55,000	50.0	--	40.0	--	10.0	--	-	--	0	
12	F	31	29,000	29,000	16.7	-	33.3	-	50.0	--	--	--	0	

\*The increase in the number of platelets in Group III at the end of eight hours is due to the fact that many of the platelets were the size of a red cell or even larger. The disintegration products of these macrothrombocytes at the end of the observation period were sufficiently large to be placed in this group.

differential formula with numerous large and giant platelets, many of them the size of a red cell. Following three blood transfusions the disintegration index dropped from 30.7 to 20.7, the total count in the meantime having risen from 127,000 to 406,000 platelets per c mm with the differential formula exhibiting a shift to the left.

*Hemophilia*—The rate of disintegration of the platelets was fairly rapid in a patient with hemophilia (Table VI) who exhibited normal total and differ-

TABLE VI  
DISINTEGRATION INDEX IN HEMOPHILIA

CASE	SEX	AGE	PLATELETS PER C MM		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D I
					GROUP I		GROUP II		GROUP III		GROUP IV		
			0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	
13	M	13	541 000	284 000	18.8	16.8	60.0	61.9	19.4	20.7	1.9	0.6	47.5

ential platelet counts. The eight hour differential formula, however, revealed relatively slight changes. In other words, the normally more resistant platelets of Group I and the less resistant platelets of the other groups all disintegrated at approximately the same rate.

It has been frequently stated that in hemophilia the platelets are resistant to disintegration (Stubel,<sup>3</sup> Pomo,<sup>37</sup> Minot and Lee,<sup>38</sup> Howell and Cekada<sup>39</sup>), the thrombocytes, being usually stable, fail to yield readily the platelet factor essential for blood coagulation. Buch<sup>40</sup> observed that by mechanically traumatizing the platelets hemophilic blood could be made to clot normally. Howell,<sup>41</sup> Minot, Denny and Davis<sup>42</sup> and Hurwitz and Lucas<sup>43</sup> noted a reduction in the

amount of prothrombin in hemophilia. This would also seem to depend on the slow disintegration of the platelets since, according to Bayne-Jones<sup>44</sup> and Tait and Green,<sup>45</sup> prothrombin and unruptured thrombocytes constitute the same substances. On the other hand, Feissly and Fried,<sup>46</sup> Fieschi<sup>47</sup> and Eagle<sup>48</sup> maintain that the hemophilic platelets are fundamentally normal. Moreover, from my own observations and from those of Baar and Székely,<sup>47</sup> it would appear that hemophilic platelets possess normal fragility. As a matter of fact there is considerable evidence indicating that a number of other factors are involved in the pathogenesis of the hemorrhagic tendencies in hemophilia. Birch,<sup>13, 49</sup> for example, found entire lack of female sex hormone in the urine of these patients. Feissly and Fried<sup>46</sup> and Fieschi<sup>47</sup> observed that in hemophilia the plasma is abnormal, since they failed to observe any difference in the coagulation time of normal plasma upon adding platelets from normal and from hemophilic subjects. Bernuth<sup>50</sup> noted that, in some cases of hemophilia, the cut capillaries failed to disappear by contraction in a normal manner, remaining visible for a long time. Stuber and Lang<sup>51</sup> found that in this disease there is an excess of fluoride in the blood which brings about a prolongation of coagulation by retarding glycolysis of the blood cells. These same investigators<sup>52</sup> also observed that in hemophilia the negative electrical charge of the platelets is increased; this causes an increase in their reciprocal electrostatic repulsion and a decrease in their rate of agglutination.

*Leucemia.*—Normal disintegration indices were observed in a patient with chronic myelogenous leucemia (Case 14, Table VII) and in one with chronic

TABLE VII  
DISINTEGRATION INDEX IN LEUCEMIAS

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
14	M	46	736,000	414,000	37.8	54.2	45.4	43.4	5.9	2.4	10.9	0	43.7	Chronic mye- logenous leu- cemia After x-ray treatment
			548,000	298,000	45.8	49.0	46.4	49.0	7.8	2.0	0.0	0	45.6	
15	F	42	252,000	217,000	32.8	40.0	50.4	54.0	16.8	6.0	0.0	0	13.8	Aleucemic myelosis
16	M	38	465,000	321,000	9.0	36.0	64.5	56.0	24.0	8.0	2.5	0	30.9	Chronic lymphatic leucemia

lymphatic leucemia (Case 16). In a patient with aleucemic myelosis (Case 15) the thrombopenia noted was associated with a rather low disintegration index.

*Malignancy.*—The disintegration index is essentially normal in malignancy (Table VIII) and does not appear to be influenced by the total or the differential platelet counts. In Cases 22 and 23, following therapeutic roentgen ray irradiation, there was a drop in the disintegration index associated with a simultaneous decrease in the total count and also a change in the differential formula characterized by a shift to the left. In a patient with chronic myelogenous

TABLE VIII  
DISINTEGRATION INDEX IN MALIGNANCY

CASE	SEX	AGE	PLATELETS PER C MM		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D I	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR		
17	M	55	638,000	459,000	47 0	64 0	34 0	33 0	7 0	3 0	12 0	0	28 0	Cancer of colon
18	M	57	721,000	493,000	16 0	47 0	49 0	47 0	35 0	6 0	0 0	0	31 6	Cancer of stomach
19	F	52	962,000	631,000	65 7	68 7	29 1	30 5	3 1	0 8	2 1	0	34 4	Cancer of stomach
20	F	50	385,000	291,000	12 0	30 0	40 0	59 0	48 0	11 0	0 0	0	24 4	Cancer of neck
21	F	59	601,000	491,000	56 0	60 0	36 8	40 0	5 6	0 0	1 6	0	18 3	Cancer of breast
22	M	60	607,000	347,000	12 4	26 0	61 4	60 0	24 8	14 0	1 4	0	42 8	Cancer of lung
23	M	38	469,000	434,000	45 1	65 0	45 9	30 0	9 0	5 0	0 0	0	7 4	After x ray treatment
			677,000	410,000	23 6	50 0	54 8	42 0	21 6	8 0	0 0	0	39 4	Hodgkin's disease
			627,000	414,000	35 5	58 0	47 8	30 0	16 7	12 0	0 0	0	34 0	After x ray treatment
			702,000	574,000	62 1	60 0	29 3	38 0	2 3	2 0	6 3	0	18 2	Two months later

leucemia (Case 14, Table VII) there was only a slight change in the disintegration index following roentgen ray irradiation. Here, however, the decrease in the total count was associated with only slight changes in the differential platelet formula.

*Infection*—In chronic infections the disintegration index is normal when the associated platelet count is normal or elevated (Cases 25, 26, 27, Table IX). The disintegration index may be low in the presence of thrombopenia (Case

TABLE IX  
DISINTEGRATION INDEX IN INFECTION

CASE	SEX	AGE	PLATELETS PER C MM		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D I	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR	0 HR	8 HR		
24	M	46	320,000	290,000	10 0	-	60 7		29 3	-	0 0	--	9 4	Acute stage of lobar pneumonia
			1,344,000	866,000	52 6	76 6	37 9	23 4	5 1	0 0	4 5	0 0	35 5	During con- valescence
			427,000	439,000	63 5	64 0	32 7	35 0	3 8	1 0	0 0	0 0	+2 8	Patient well
25	M	55	611,000	239,000	62 5	80 0	31 0	20 0	1 9	0 0	4 6	0 0	60 0	Bronchec- tasis
26	F	40	658,000	472 000	57 8	80 0	37 1	20 0	3 1	0 0	3 1	0 0	28 2	Active pul- monary tu- berculosis
27	M	31	478,000	366,000	26 3	30 0	50 0	62 0	23 7	8 0	0 0	0 0	23 4	Subacute bacterial endo- carditis
28	M	21	204 000	199,000	50 0	56 0	40 0	38 0	6 0	0 0	6 0	2 4	2 4	Subacute bacterial endo- carditis

28). In acute infections (Case 24, Table IX) the disintegration index is low when thrombopenia exists, rises during convalescence when thrombocytosis appears, and then decreases as the normal level is reached. During the latter stage the slow disintegration of the platelets is apparently due to the presence of relatively numerous young, resistant thrombocytes as manifested by the differential formula.

*Postoperative Behavior of the Disintegration Index.*—Following surgical operations there is usually a decrease in the total platelet count during the first twenty-four hours. This is followed by a gradual increase in the number of platelets reaching a maximum level toward the end of the second week, and then gradually returning to normal. The entire cycle occupies about three weeks. The disintegration index exhibits a corresponding swing (Table X,

TABLE X  
POSTOPERATIVE BEHAVIOR OF DISINTEGRATION INDEX

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
29	F	41	342,000	305,000	43.0	65.0	51.0	33.0	6.0	2.0	0.0	0.0	10.8	Cholecystec- tomy 24 hours post- operatively  Sixteenth postopera- tive day
			879,000	600,000	59.2	71.8	30.2	21.2	7.0	4.0	3.3	3.3	31.7	
30	F	45	536,000	486,000	78.0	80.0	20.9	19.5	1.1	0.5	0.0	0.0	9.5	Amputation of foot 12 hours post- operatively  Thirteenth postoperative day
			901,000	513,000	69.2	80.0	22.8	16.0	4.0	2.0	2.0	1.0	43.1	

Chart 3). It is low during the early postoperative period, rises as the total platelet count increases, then decreases as the normal platelet level is reached. During the stage of thrombocytosis more than half of all the platelets belong to Group I. However, despite the presence of many young, relatively resistant platelets, the disintegration index is fairly high. This is probably due to the fact that the remaining platelets are unusually fragile. These observations are in accord with those of König<sup>23</sup> who noted that postoperatively the platelets, owing to the damaging effect of the spleen, disintegrate more readily than normally.

The rate of disintegration of the platelets is probably a factor of importance in the causation of spontaneous venous thrombosis as observed postoperatively and also during the course of other diseases. Spontaneous venous thrombosis is nearly always associated with an elevated platelet count in conditions such as postoperative states (Hueck<sup>53-55</sup>), fractures (Galloway<sup>56</sup>), parturition (Dawbarn, Earlam and Evans<sup>57</sup>), severe acute hemorrhage (Jagic and Klima<sup>58</sup>), infection (Brock and Rake<sup>59</sup>), certain splenic anemias (Rosenthal<sup>60</sup>), malignancy (Naegeli<sup>61</sup>), and polycythemia vera (Jürgens and Bach<sup>62</sup>). Essential thrombo-

philia described by Nygaard and Brown<sup>60</sup> constitutes an exception, for in this disease the platelet count is normal. The characteristic features of this thrombocytosis are moderate fragility and increased agglutinability of the platelets. It is, therefore, probable that the rapid disintegration of the thrombocytes plays an important rôle in the pathogenesis of spontaneous intravascular clotting. In fact, König<sup>23</sup> maintains that the decreased stability of the platelets is a very significant predisposing factor favoring the development of spontaneous venous thrombosis and embolism during postoperative states.

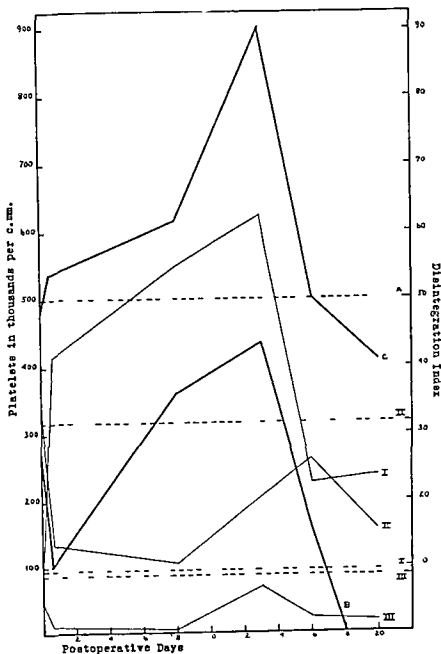


Chart 3—Behavior of platelets postoperatively (follow normal platelet level groups). Continuous lines I, II, and III represent the behavior of the

differential platelet formula postoperatively (see Table X). Dotted line A represents the normal absolute levels of the corresponding groups. Continuous line B represents

**Miscellaneous Diseases**—The fragility of the platelets was normal in a patient with purpura rheumatica (Case 31, Table XI), and in one with hemochromatosis (Case 40). The latter patient presented an extremely abnormal differential formula due to the presence of numerous large and giant platelets. The disintegration index was normal in patients with jaundice (Cases 35, 36, 37, 38, 39, Table XI) despite the frequently encountered thrombopenia asso-

ciated with an abnormal differential formula revealing a shift to the left. The platelets appeared to be abnormally resistant to disintegration in a patient with senile purpura (Case 32), in one with scurvy (Case 33), and in one with malignant nephrosclerosis (Case 34). The last patient presented numerous spontaneous hemorrhages from the mucous membranes of the mouth and skin.

TABLE XI  
DISINTEGRATION INDEX IN MISCELLANEOUS DISEASES

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.				
31	F	32	471,000	285,000	15.3	29.0	70.3	61.0	13.7	10.0	0.7	0.0	39.5	Purpura rheumatica
32	M	70	355,000	415,000	9.3	37.0	42.6	53.0	47.4	10.0	0.7	0.0	+17.0	Senile purpura
33	F	50	423,000	410,000	70.9	70.0	25.2	28.0	3.9	2.0	0.0	0.0	3.0	Scurvy (partly treated)
34	F	30	300,000	287,000	24.0	38.0	47.0	50.0	27.0	8.0	2.0	4.0	4.3	Malignant nephrosclerosis
35	F	35	255,000	157,000	36.6	43.0	35.8	50.0	24.4	7.0	3.2	0.0	38.4	Catarrhal jaundice
			437,000	371,000	12.7	14.0	68.6	70.0	18.7	16.0	0.0	0.0	15.1	Patient well
36	M	31	402,000	237,000	61.8	56.0	35.2	40.0	3.0	4.0	0.0	0.0	41.0	Catarrhal jaundice
37	M	30	345,000	268,000	25.0	39.0	56.0	49.0	19.0	12.0	0.0	0.0	22.3	Catarrhal jaundice
38	M	23	406,000	293,000	31.0	28.0	41.0	54.0	28.0	8.0	0.0	0.0	27.8	Chronic hemolytic jaundice
39	F	28	367,000	279,000	33.0	50.0	53.0	45.0	14.0	5.0	0.0	0.0	31.5	Jaundice due to ar-sphenamine hepatitis
40	M	59	257,000	174,000	4.5	18.0	39.1	48.0	54.3	34.0	2.1	0.0	32.3	Hemo-chromatosis

#### EFFECT OF ROENTGEN RAY IRRADIATION ON THE BLOOD PLATELETS

Observations on the effect of roentgen ray irradiation on the circulating blood platelets have yielded rather confusing results. Helber and Linser<sup>63</sup> and Haenisch and Holthusen<sup>64</sup> maintain that roentgen rays have a slight effect on the platelets. Gaviati,<sup>65</sup> on the other hand, is of the opinion that the platelets are very sensitive to x-ray irradiation. Falconer, Morris, and Ruggles<sup>66</sup> noted that direct x-ray irradiation of the long bones, in both light and heavy dosage, did not appreciably stimulate or decrease the contained marrow cells. According to Wittkower<sup>67</sup> there may be an increase or decrease in the number of circulating platelets after x-ray irradiation. Henkel and Gueffroy<sup>68</sup> observed no change in the number of platelets after roentgen ray exposure. According to Minot and Spurling<sup>69</sup> in malignancy medium x-ray doses cause a slight increase in the number of circulating platelets, larger doses a great increase even up to 100 per cent, larger doses still after an initial increase produce a decrease, yet the count remains above normal. Hittmair, Luze and Hönlinger<sup>70</sup> noted an increase in the platelet count to over a million per cubic millimeter following

irradiation for lymphogranulomatosis. Many investigators (Falconer, Morris and Ruggles,<sup>66</sup> Schintz,<sup>71</sup> Hittmair<sup>72</sup> among others) have observed an increase in the number of platelets following irradiation of the spleen. I have failed to observe this reaction in two patients with purpura hemorrhagica. On the other hand, severe thrombopenias may be produced in animals by exposing them to roentgen rays (Helber and Linser,<sup>63</sup> Duke,<sup>73</sup> Fabricius-Möller<sup>74</sup>). In human subjects decreases in the platelet count after x-ray irradiation have been observed by Schintz,<sup>71</sup> Frei and Alder,<sup>75</sup> and Hirschfeld and Hittmair.<sup>76</sup> Apparently the biologic reactions following exposure to radium are essentially the same (Cramer, Drew and Mottram<sup>77</sup>).

There is no doubt that the reaction on the part of the platelets depends on the condition of the patient, the type of apparatus employed and the amount of irradiation. One very significant factor is the absolute platelet level. There is nearly always a reduction in the number of circulating platelets following therapeutic doses of roentgen ray irradiation in patients presenting moderate or marked thrombocytosis. I have observed this reaction in malignancy, Hodgkin's disease, chronic myelogenous leucemia, and polycythemia vera.

No observations are recorded in the literature concerning the effect of x-ray irradiation or radium on the fragility of platelets. From my own observations, it would appear that the rate of disintegration of the platelets is decreased following therapeutic roentgen ray irradiation whenever there is a shift of the differential formula to the left due to an increase in the number of small platelets. These observations also suggest that the larger, more mature platelets are more sensitive to roentgen rays than the smaller thrombocytes.

#### SUMMARY AND CONCLUSIONS

1. A method is described for the simultaneous determination of the rate of disintegration, total and differential counts of the platelets.

2. The large, more mature platelets are less stable and disintegrate more readily than the small, juvenile thrombocytes.

3. The rate of disintegration of the platelets varies considerably in normal persons. In a group of 26 normal individuals the following results were obtained: in 50 per cent of the subjects, 21 to 40 per cent of all the platelets disintegrated in the course of eight hours, and in 85 per cent, 11 to 60 per cent of the platelets became completely disintegrated during the observation period. Abnormal stability of the platelets is indicated by a slow disintegration rate when 10 per cent or less of all the thrombocytes become disintegrated during the observation period. Abnormally rapid disintegration rates, when 60 per cent or more of the platelets disintegrate in the course of eight hours, are very infrequent in normal or abnormal states.

4. The rate of disintegration of the platelets was studied in a variety of diseases. In chronic conditions such as anemia, at times essential purpura, purpura rheumatica, hemophilia, polycythemia vera, leucemia, malignancy, jaundice, hemochromatosis, infection and postoperative states the fragility of the platelets was normal and did not appear to be influenced by the total thrombocyte level or the differential platelet formula. As the total platelet count changed there was a simultaneous change in the stability of the platelets usu-

ally associated with a shift of the differential formula to the left. As a rule the fluctuations remained within normal limits. However, it was the relative variations in the rate of disintegration of the platelets that appeared to be of significance. A change in the rate of the disintegration of the thrombocytes from 9.5 per cent to 43.1 per cent (Case 30, Table X), for example, indicates a change in the resistance of the platelets from a state of moderate stability to one of moderate fragility despite the fact that these values fall within the normal range. In thrombopenic states the rate of disintegration of the platelets decreased as the total platelet count rose to the normal level. In conditions associated with thrombocytosis (infective and postoperative states) the fragility of the platelets increased as the total platelet count rose and then decreased as the normal platelet level was reached. The increase in the fragility of the platelets during infective and postoperative states, though relative, is probably of considerable significance in the pathogenesis of spontaneous venous thrombosis at times observed in these conditions.

5. Abnormal stability of the platelets was noted in marked and occasionally in moderate thrombopenias, in scurvy, senile purpura, malignant nephrosclerosis, polycythemia vera treated with phenylhydrazine, and at times in conditions with normal platelet counts following a recent preexisting thrombocytosis (infective and postoperative states). This abnormally high resistance of the platelets was frequently associated with spontaneous hemorrhages.

6. Following therapeutic roentgen ray irradiation there was an increase in the resistance of the platelets to disintegration associated with a simultaneous decrease in their number and a change in the differential formula characterized by a shift to the left due to an absolute increase in the number of the smaller thrombocytes.

#### REFERENCES

1. Irish, D. D.: A New Method for Determining the Fragility of Blood Platelets, *J. Biol. Chem.* 100: Lvi, 1933.
2. Mayer, A.: La Coagulation du Plasma Sanguin: Étude Ultramicroscopique, *Compt. rend. Soc. de biol.* 63: 658, 1907.
3. Stübel, H.: Ultramikroskopische Studien über Blutgerinnung und Thrombocyten, *Pflüger's Arch. f. d. ges. Physiol.* 156: 361, 1914.
4. Tait, J., and Burke, H. E.: Platelets and Blood Coagulation, *Quart. J. Exper. Physiol.* 16: 129, 1927.
5. Ferguson, J. H.: Observations on the Alterations of Blood Platelets as a Factor in Coagulation of the Blood, *Am. J. Physiol.* 108: 670, 1934.
6. Flössner, O.: Beobachtungen und Zählung von Blutplättchen, *Ztschr. f. Biol.* 77: 113, 1922-1923.
7. Hofmann, F. B.: Ueber Blutplättchenzählung, *Deutsche med. Wchnschr.* 52: 861, 1926.
8. Boshamer, K.: Ueber Zählung, Resistenz und Neubildung von Blutplättchen, *Ztschr. f. d. ges. exper. Med.* 48: 631, 1925-1926.
9. Horwitz, S.: Die klinische Bedeutung der Blutplättchenzählmethode nach Hofmann-Flössner, *Ztschr. f. d. ges. exper. Med.* 57: 380, 1927.
10. Horwitz, S.: Neuere Untersuchungen an Blutplättchen, *Ztschr. f. d. ges. exper. Med.* 73: 422, 1930.
11. Horwitz, S.: Die Blutplättchen in methodischer Hinsicht, *Klin. Wchnschr.* 10: 1613, 1931.
12. Tyrode, M. V.: The Mode of Action of Some Purgative Salts, *Arch. internat. de pharmac. et de therap.* 20: 205, 1910.
13. Birch, C. L.: Hemophilia, *Proc. Soc. Exper. Biol. & Med.* 28: 752, 1930-1931.
14. Kugelmass, I. N.: The Management of Hemorrhagic Problems in Infancy and Childhood, *J. A. M. A.* 99: 895, 1932.
15. Aynaud, M.: Le globulin des mammifères, Thèse, Paris, 1909.

- 16 Aynaud, M    Le globulin de l'homme, Ann de l'Inst Pasteur 25 56, 1911
- 17 Bar, H, and Székely L    Ueber die Plattchenzeifallsgeschwindigkeit bei normalen Kindern, bei der Hämophilie und Thrombopenie Ztschr f Kinderheilk 48 31, 1929
- 18 Bar, H, and Canaval Székely, L    Ueber die Plattchenzeifallsgeschwindigkeit, Klin Wehnschr 10 2045, 1931
- 19 Bar, H    Zur Kenntnis der Blutungsnebel im Kindesalter, Wien Klin Wehnschr 47 1039, 1934
- 20 Kiguchi, N, and Mizuta, S    The Speed of Platelet Destruction in Citrated Blood, Mitt a d med Akad zu Kyoto 9 703, 1933
- 21 Preiss, W    Ueber die physiologische Zahl und Morphologie der Blutplättchen, Ztschr f d ges exper Med 84 810, 1932
- 22 Aynaud, M    Action du metaphosphate de soude sur les globulins, Compt rend Soc de biol 68 916, 1910
- 23 König, W    Experimentelle Untersuchungen über die Entstehung der Thrombose, Arch f klin Chir 171 447, 1932
- 24 Kristenson A    A New Method for the Direct Counting of Blood Platelets, Acta med Scandinav 57 301, 1922
- 25 Pratt J H    Beobachtungen über die Gerinnungszeit des Blutes und die Blutplättchen, Arch f exper Path u Pharmac 49 299, 1902 1903
- 26 Pratt, J H    A Critical Study of the Various Methods Employed for Enumeration of Blood Platelets J A M A 45 1999, 1903
- 27 Pratt J H    Observations on the Coagulation Time of the Blood and Blood Plates J Med Res 10 120, 1903
- 28 Morawitz, P    Beiträge zur Kenntnis der Blutgerinnung, Deutsches Arch f klin Med 79 215, 1901 1904
- 29 Schittenhelm, A and Bodong A    Beiträge zur Frage der Blutgerinnung mit besonderer Berücksichtigung der Hirudinwirkung, Arch f exper Path u Pharmac 54 217, 1906
- 30 Abderhalden, F, and Deetjen H    Weitere Studien über den Abbau einiger Polypeptide durch die roten Blutkörperchen und die Blutplättchen des Pferdeblutes Hoppe Seyler's Ztschr f physiol Chem 53 280 1907
- 31 Burkner, K    Blutplättchen und Blutgerinnung, Arch f d ges Physiol 102 36, 1904
- 32 Olef, I    The Enumeration of Blood Platelets, J LAB & CLIN MED 20 416, 1935
- 33 Olef, I    The Differential Platelet Count Its Clinical Significance, Arch Int Med 57 1163, 1936
- 34 Morawitz H and Pratt, J H    Einige Beobachtungen bei experimentellen Anämien München med Wehnschr 55 1817 1908
- 35 Itami, S, and Pratt J H    Ueber Veränderungen der Resistenz und der Stromatoroter Blutkörperchen bei experimentellen Anämien Biochem Ztschr 18 302, 1909
- 36 Jürgens P and Buch K    Thrombosenbereitschaft bei Polythaemia Vera, Deutsches Arch f klin Med 176 726, 1934
- 37 Fomic A    Ueber die Gerinnungsfaktoren des Hämophilen Blutes, Mitt a d Grenzgeb d Med u Chir 28 313 1914 1915
- 38 Minot G R and Lee, R I    The Blood Platelets in Hemophilia, Arch Int Med 18 474 1916
- 39 Howell W H and Calkins F B    The Cause of the Delayed Clotting of Hemophilic Blood Am J Physiol 78 500 1926
- 40 Birch, C L    Hemophilia, J A M A 99 1506 1932
- 41 Howell, W H    The Condition of the Blood in Hemophilia Thrombosis and Purpura, Arch Int Med 13 76 1914
- 42 Minot, G R Denny, G P and Davis, D    Prothrombin and Antithrombin Factors in the Coagulation of Blood Arch Int Med 17 101, 1916
- 43 Hurwitz, S H and Lucas W P    A Study of Blood in Hemophilia, Arch Int Med 17 543, 1916
- 44 Bayne Jones, S    The Presence of Prothrombin and Thromboplastin in Blood Platelets, Am J Physiol 30 74, 1912
- 45 Tait J, and Green F    The Spindle Cells in Relation to Coagulation of Frog's Blood Quart J Exper Physiol 16 141, 1927
- 46 Lessly R and Fried, A    Die Blutplättchen des Hämophilen Blutes, Klin Wehnschr 3 831, 1924
- 47 Pleschi A    Ricerche sull emofilia Clin med Ital 63 560, 1932, Abst Folia haemat 52 226, 1934
- 48 Lingle H    Studies on Blood Coagulation IV The Nature of the Clotting Deficiency in Hemophilia J Gen Physiol 18 813 1935
- 49 Birch, C L    Hemophilia and the Female Sex Hormone, J A M A 97 244, 1931

50. Bernuth, F.: Ueber Kapillarbeobachtungen bei Hämophilie und anderen hämorrhagischen Diathesen, Deutsches Arch. f. klin. Med. 152: 321, 1926.
51. Stuber, B., and Lang, K.: Untersuchungen zur Lehre von der Blutgerinnung, Biochem. Ztschr. 212: 96, 1929.
52. Stuber, B., and Lang, K.: Zur Pathogenese und Therapie der Thrombose, Klin. Wchnschr. 9: 1113, 1930.
53. Hueck, H.: Ueber Untersuchungen der Eiweisskörper des Blutes, sowie Blutplättchen-zählungen, besonders nach Operationen, Deutsche med. Wchnschr. 51: 1869, 1925.
54. Hueck, H.: Blutplättchen-Untersuchungen bei chirurgischen Erkrankungen, Deutsche Ztschr. f. Chir. 192: 322, 1925.
55. Hueck, H.: Blutplättchenveränderungen nach Operationen, München. med. Wchnschr. 73: 173, 1926.
56. Galloway, J. F.: The Blood Platelets After Fracture, Lancet 1: 1082, 1931.
57. Dawbarn, R. Y., Earlam, F., and Evans, W. H.: The Relation of the Blood Platelets to Thrombosis After Operation and Parturition, J. Path. 31: 833, 1928.
58. Jagie, N., and Klima, R.: Klinik und Therapie der Blutkrankheiten, ed. 2, Berlin und Wien, 1934, Urban und Schwarzenberg.
59. Brock, R. C., and Rake, G. W.: Some Observations on Blood Platelets, Guy's Hosp. Rep. 9: 451, 1929.
60. Rosenthal, N.: Clinical and Hematological Studies on Banti's Disease. I. The Blood Platelet Factor With Reference to Splenectomy, J. A. M. A. 84: 1887, 1925.
61. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, 1931, Julius Springer.
62. Nygaard, K. K., and Brown, G. E.: Essential Thrombophilia, Proc. Staff Meet. Mayo Clinic 10: 13, 1935.
63. Helber, E., and Linser, P.: Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf das Blut, München. med. Wchnschr. 52: 689, 1905.
64. Haenisch, G. F., and Holthausen, H.: Einführung in die Röntgenologie, Leipzig, 1933, Georg Thieme.
65. Gaviati, A.: Sulle alterazioni morfologiche e degenerative del sangue di animali sottoposti ai raggi X, studiate col metodo della colorazione vitale, Haematologica 1: 273, 1920.
66. Falconer, E. H., Morris, L. M., and Ruggles, H. E.: The Effects of X-rays on Bone Marrow, Am. J. Roentgenol. 11: 342, 1924.
67. Wittkower, E.: Klinische und experimentelle Untersuchungen zur Blutplättchen-frage, Ztschr. f. d. ges. exper. Med. 25: 73, 1921; *ibid.* 26: 250, 1922.
68. Henkel, M., and Gueffroy, H.: Blutgerinnung bei Röntgentiefentherapie, Zentralbl. f. Gynäk. 46: 409, 1922.
69. Minot, G. R., and Spurling, R. G.: The Effect on the Blood of Irradiation, Especially Short Wave Length Roentgen-Ray Therapy, Am. J. M. Sc. 168: 215, 1924.
70. Hittmair, A., Luze, O., and Hönlinger, H.: Ueber Lymphogranulomatose, Wien. klin. Wchnschr. 37: 159, 1924; *ibid.* 37: 190, 1924.
71. Schintz, H. R.: Blutungszeit und Röntgenbestrahlung, Arch. f. klin. Chir. 132: 402, 1924.
72. Hittmair, A.: Die Blutplättchen, Folia haemat. 35: 156, 1927-1928.
73. Duke, W. W.: Variation in the Platelet Count: Its Cause and Clinical Significance, J. A. M. A. 65: 1600, 1915.
74. Fabricius-Möller, J.: Étude expérimentales sur la diathèse Hémorrhagique déterminée par les rayons roentgen, Compt. rend. Soc. de biol. 87: 759, 1922.
75. Frei, C., and Alder, A.: Einfluss der Röntgenstrahlen auf Blut- und Agglutininbildung, Schweiz. med. Wchnschr. 5: 670, 1924.
76. Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin & Wien, 1932, Urban & Schwarzenberg, vol. 1.
77. Cramer, W., Drew, A. H., and Mottram, J. C.: On Blood Platelets: Their Behavior in "Vitamin A" Deficiency and After "Radiation," and Their Relation to Bacterial Infections, Proc. Roy. Soc. London, Ser. B. 93: 449, 1921-1922.

# THE VALUE OF AIR CONDITIONED ROOMS IN THE TREATMENT OF SEASONAL AND PERENNIAL ASTHMA\*

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AIR conditioning has decided therapeutic values. This has been proved by many investigators in the field of Sanitary Engineering, and accepted by medical authorities. Its application is not only limited to the sickroom or the hospital, but it offers comfort for the healthy one.

The purpose of this presentation is to record our observations of the effects in the treatment of seasonal and nonseasonal asthma.

The Mount Sinai Hospital has two rooms equipped with built in room type units. Each room contains four beds about 3,200 cu. ft. of air space.

The units which are classed as HC600 units were built and put in operation April, 1934. Each unit is capable of delivering 400 cubic feet of air per minute at a dry bulb temperature of 60°, a dew point of 55° and of maintaining a room temperature at 80° dry bulb and 67° wet bulb with outside conditions 95° dry bulb and 77° wet bulb. We maintain a room temperature of from 73 to 74° dry bulb and from 64 to 66° wet bulb. This gives a relative humidity of 63 per cent with eight complete air changes per minute. The operation of the unit is as follows: There is 20 per cent air drawn in from the outside which passes to the bottom where it mixes with the air being drawn in from the room. This mixed air passes up through refrigeration coils where it is cooled and dehumidified and then through a filter which removes pollen and dust, and then through a fan which circulates the air over steam coils. Then the air is blown into the room. The refrigeration and steam are automatically controlled by solenoid valves, thermostat and humidstat, and the air is humidified in winter by the air passing through a water spray. The compressors which are installed in the basement have a capacity of one and one half tons of refrigeration and are charged with a compound known as F12 or Freon which is dichlorodifluoromethane and is nonpoisonous, colorless, odorless, and noncombustible except under extreme temperature. The compressor is driven by a two horse power motor.

All of our asthmatics are usually admitted to these wards, where they are carefully observed and studied. The number of admissions during this period totals 40.

For practical purposes our cases can be divided into three groups: (1) Pollen asthma, (2) perennial asthma, (3) mixed group, i.e., perennial asthmatics who have definite seasonal aggravation usually due to pollen.

Our pollen asthma cases totaled 10, 7 ragweed and 3 timothy. One case, while seasonal in incidence, had no definite etiology.

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During the summer of 1934, we had 3 cases, and in 1935, 8 cases. All were asthmatics. Two also had nasal symptoms. Eight did not receive any pre-seasonal treatment. Two began coseasonal treatment and failed to get any relief within one week. They were admitted in the height of the season, and all of them were in a very severe form of "status asthmaticus." All of them, except one, showed evidence of improvement within two to three hours after admission, and marked improvement within twenty-four hours. One of the cases was practically moribund. She was admitted late on the night of September 4. During ward rounds about 10 A.M. the following morning, she was very comfortable, although her chest was still barrel-shaped and filled with various musical râles.

The one exceptional case that failed to respond in 1934 and 1935 is quite a puzzle to us. He is an Armenian of about fifty-two years, who is engaged in the industry of cleaning and repairing of rugs. His duties, however, are limited to collecting and delivering only. His symptoms are of twenty years' duration, and are always limited to July and the early part of August. Skin tests and conjunctival tests were negative to all of the known midsummer pollens. The only moderate reaction obtained was to house dust. We were unsuccessful in bringing on an attack of asthma by introducing powdered pollen into the nasal chamber and conjunctival sac. The clinical course is usually associated with fever, ranging from 100 to 101° F., and the physical signs, in addition to the typical picture of asthma, areas of bronchopneumonia; x-ray of the chest on three different occasions failed to reveal any consolidation. It was not associated with hemoptysis. Sputum examination revealed a number of eosinophiles. The blood examination revealed a leucocytosis of about 15,000 with a normal differential containing 3 or 4 per cent eosinophiles.

What the cause of this seasonal asthma is remains undecided.

Kahn and Grothaus<sup>1</sup> feel that air-conditioned rooms have not only a therapeutic but also a diagnostic value. They feel that when relief in air-conditioned rooms is obtained in patients who give negative skin reactions to pollen, they call it "cutaneous anergy," it is proof that pollen was the cause of the asthma. If the reverse is true, then our exceptional case is seemingly not a pollen asthmatic.

In order to demonstrate that the beneficial results in the above cases were due to the air-conditioning, may we cite the cases of two patients admitted to the private service of A. T. in the summer of 1933.

These two patients were victims of timothy allergy. Both were untreated and in "status asthmaticus." They were admitted about the middle of July. It took more than twenty-four hours before their attacks could be ameliorated, and then they never remained symptom-free, requiring epinephrin and other similar medications to control frequent attacks. While we made no pollen counts, we recollect definitely that during a rainy day they would invariably feel better, and attacks would recur whenever wind and sunshine prevailed.

*The "mixed group" consists of seven patients.* It may be of interest to note that two began with seasonal asthma, and one with seasonal hay fever before they developed perennial symptoms. All of them had preseasonal pollen treatment as well as treatment for the perennial factors without any relief. They were admitted during the latter part of their respective pollination season, and

all of them were in "status asthmaticus" No relief was noted within twenty-four hours, and in a few, in forty eight hours Only one patient, who had a rather mild form of perennial asthma, developed very severe symptoms during the timothy season He was relieved within a few hours after admission, so that all medications were discontinued after the first day of hospitalization He was discharged at the end of July, and enjoyed good health until the middle of September, when he contracted an acute upper respiratory infection, resulting in a severe form of asthma Readmission this time did not yield any dramatic results Improvement of his asthma followed the improvement of his upper respiratory infection, which took about two or three days

*The perennial asthma group consists of 22 cases* They reacted to various inhalants and ingestants A few had chronic pathology in the paranasal sinuses Five of this group were admitted in "status asthmaticus" The remainder were admitted either for additional studies, nasal surgery, or to observe the effects of environmental changes

The immediate effects were not dramatic It took twenty four and forty eight hours before any relief was definitely obtained Those in "status asthmaticus" had to have epinephrine every three hours, and other emergency measures in order to be free from attacks The others had to have nightly administration of epinephrine All of them, however, experienced some relief within a few days

Four patients who were admitted to the general wards experienced just as much relief within two days Two patients, one a woman of sixty years, and the other twenty eight, were worse in the air conditioned room and begged to be removed to the general ward

This again is in conformity with the experience of Gay,<sup>2</sup> Nelson, Rappoport and Walker<sup>3</sup>

#### COMMENT

Our results confirm the observations of Gay who was able to obtain favorable results in his asthmatic patients within one hour after their admission to the air conditioned room, and that of Nelson, Rappoport and Walker, who report 50 per cent improvement in their asthmatic patients within three hours or less While the bulk of their patients consisted of hay fever cases whose symptoms were referable to the upper respiratory tract, we feel that the asthmatic patients surely present a much more urgent problem in treatment, hence, the need of air conditioning is greater

It is also evident from our results that only certain extrinsic forms of asthma are likely to benefit from this method of treatment One should not expect to obtain relief in such an atmosphere when sensitivity exists to the animal danders, which are contained in that room This is well exemplified in the case of Vaughan and Cooley,<sup>4</sup> whose patient was sensitive to feathers, and failed to improve in the air conditioned room which contained feathers Intrinsic factors are not controlled nor removed As in the case of Gay, patients suffering from bacterial allergy cannot expect any benefit in such rooms In fact, they are frequently made very uncomfortable This explains the failure of treatment in the perennial as well as mixed asthmatic patients in air conditioned atmospheres

Where environmental factors exist, one does not need air-conditioned rooms. Such patients will get just as much relief in the general wards. It is our experience, and we are sure, that of every allergist, that we obtain marked relief in such patients upon their admission to the hospital, and that they stay well while in the hospital, and develop seizures upon returning to their old environment.

We are inclined to agree with Kahn and Grothaus that the air-conditioned room has some diagnostic value. Failure to obtain immediate relief in such an atmosphere would justify the exclusion of pollen as a factor.

#### SUMMARY

1. Air-conditioned atmosphere has a definite value in the treatment of seasonal asthma, due to pollen.

2. It is useless in other forms of asthma, in fact, it may aggravate cases of bacterial allergy.

3. The improvement obtained in the nonseasonal asthmatic is usually due to the removal of some environmental factors to which the patient is sensitive. Such results can easily be obtained in the general wards which are not air-conditioned.

#### REFERENCES

1. Kahn, I. S., and Grothaus, E. M.: Antigen-Free Room in the Detection and Control of Cutaneous Anergy in Pollen-Bronchial Asthma, *J. Allergy* 5: 45, 1933.
2. Gay, Leslie, N.: The Treatment of Hay-Fever and Pollen-Asthma by Air-Conditioned Atmosphere, *J. A. M. A.* 100: 1382, 1933.
3. Nelson, T., and Rappoport, B. B., and Walker, Wm. H.: The Effect of Air Filtration in Hay-Fever and Pollen-Asthma, *J. A. M. A.* 100: 1385, 1933.
4. Vaughan, Warren T., and Cooley, L. E.: Air-Conditioning as a Means of Removing Pollen and Other Particulate Matter, and of Relieving Pollinosis, *J. Allergy* 5: 37, 1933.

# PRIMARY CARCINOMA OF THE THYMUS GLAND\*

## CASE REPORT

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PRIMARY carcinoma of the thymus gland is a relatively rare tumor Crosby,<sup>1</sup> in an exhaustive review of the literature on thymogenic tumors, was able to collect reports on 44 cases of thymic carcinoma out of a total of 166 cases of primary thymic tumors. We have been able to find reports of 9 other cases since Crosby made his summary. Symmers<sup>2</sup> reported 2 cases. Leyton, Turnbull, and Bratton<sup>3</sup> reported 2 cases. McDonald,<sup>4</sup> Demel,<sup>4</sup> Touriff, and Collins<sup>5</sup> each reported 1 case. These, together with our own case, bring the total number up to 53.

Symmers<sup>2</sup> subdivides thymic tumors into the following groups, in order of their frequency: (1) Lymphosarcoma, (2) perithelioma, (3) Hodgkin's disease, (4) epithelioma, and (5) spindle cell sarcoma. He reported a total of 25 primary thymic tumors, of which 2 were epitheliomas.

Ewing<sup>7</sup> gives the following classification: (1) Lymphosarcoma or thymoma consisting of a diffuse growth of round, polyhedral, and giant cells, (2) carcinoma, and (3) spindle cell sarcoma or myxosarcoma.

The occurrence of epithelial tumors of the thymus gland can be explained on an embryologic basis. The gland has its origin from the entodermal pouches that arise from the third and fourth branchial clefts. The reticulum cells and Hassal's corpuscles are of epithelial origin. The epithelioma may arise either from Hassal's corpuscles or from the reticulum cells.

The clinical picture of all thymic tumors is the same. There is nothing to distinguish a carcinoma from a lymphosarcoma clinically, except that the latter sometimes responds to radiotherapy.

The subjective symptoms are those of pressure in the upper portion of the mediastinum. Cough, hoarseness, dyspnea, cyanosis, and edema of the neck and face are the chief symptoms. Exophthalmos is frequently encountered. The veins of the neck become distended. There may be some difficulty in swallowing.

Physical examination may reveal retrosternal dullness. A pericardial effusion is sometimes encountered. X-ray examination shows a tumor which is located in the upper portion of the anterior mediastinum.

The symptoms usually do not appear until quite late. In most of the cases that have been reported, the patient did not seek any medical advice until a few weeks or at the most a few months before death occurred. In our case the

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patient consulted a physician about two months before he died, and he stated that he had been perfectly well until about four weeks before that time.

A peculiar feature of some thymic tumors, particularly of the lymphosarcomas, is the similarity of the symptoms to those of myasthenia gravis. Several cases of this type have been reported. Leyton, Turnbull, and Bratton<sup>8</sup> reported 2 cases with pluriglandular disturbances; both of these cases were carcinomas.

The treatment of thymic tumors is unsatisfactory. Some of the lymphosarcomas and some of the cases of Hodgkin's disease respond, at least temporarily, to x-ray treatment. Carcinoma of the thymus gland is not radiosensitive.

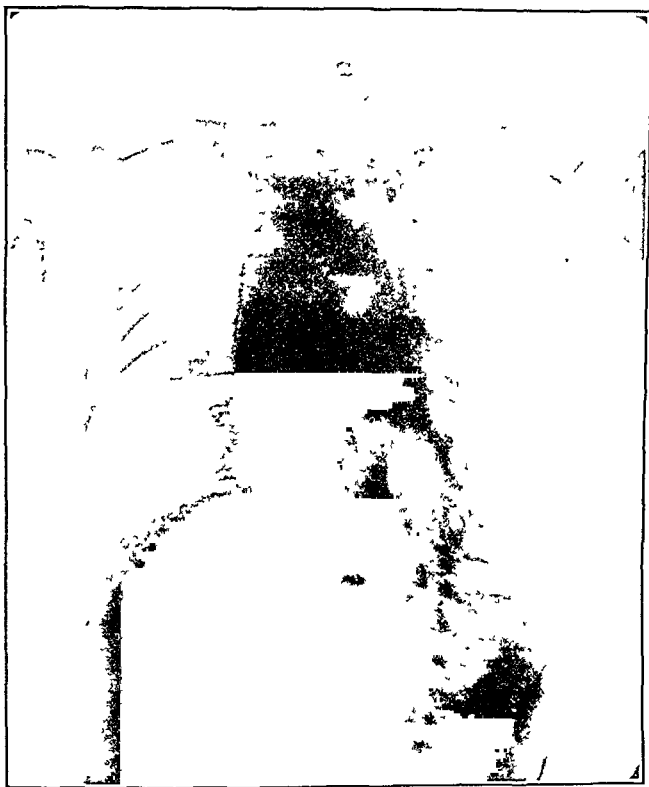


Fig. 1.—Roentgenogram of chest.

#### REPORT OF CASE

F. M., white, male, aged forty-nine years. Admitted to the Windber Hospital on Oct. 30, 1934. Chief complaints were dyspnea and swelling of face and neck. He stated that he had been perfectly well until Oct. 1, 1934. At that time he noticed a moderate degree of dyspnea. About one week later he noticed some puffiness of his neck. The dyspnea became worse and he came to the hospital on October 30.

*Physical Examination.*—Patient was well nourished. There was a moderate degree of swelling of the face and neck. The lips were somewhat cyanotic. There was moderate exophthalmos of both eyes. The pupils were equal and reacted to light and accommodation. The external jugular veins were prominent on both sides. The respiratory excursion was diminished bilaterally. There was dullness to the right of the sternum. The heart sounds were distant.

*X-ray Examination*—X-ray of the chest showed a large shadow in the upper portion of the mediastinum

A diagnosis of mediastinal tumor was made, and the patient was given a course of deep x-ray treatments. However, he did not seem to respond to the treatment. His condition gradually became worse. He developed marked hoarseness. The edema of the face and neck became worse. Cyanosis became marked. He also developed marked edema of the scrotum and lower extremities. Death occurred on Dec 20, 1934.

*Autopsy*—The body was that of a well nourished middle aged male. The face, neck, scrotum, and lower extremities were edematous. Both eyes showed a moderate degree of



Fig 2—Gross appearance of tumor showing relationship to heart and lungs



Fig 3—Gross appearance of tumor

exophthalmos. The superficial veins of the neck were prominent. Upon opening the thoracic cavity, a large tumor was found in the upper portion of the anterior mediastinum. It occupied the position which is normally assigned to the thymus gland, and its contour was that of a large thymus gland, consisting of two lobes, the lower ends of which were separated by a notch. The tumor extended from the manubrium sterni to a point between the fourth and fifth ribs. It was extremely hard and was adherent to the surrounding tissues.

The pericardium contained about 125 cc of a straw colored fluid. There were adhesions of the pleura at both apices. Both lungs showed a marked degree of pneumoconiosis. The azygos vein was markedly dilated as were also the pulmonary veins and pulmonary artery. There was a large thrombus in the right auricle. The peribronchial lymph nodes were en-

larged and hardened. Abdominal examination was essentially negative. There was no free fluid in the peritoneal cavity. There was no evidence of metastasis of the tumor to any of the abdominal organs.

*Description of the Tumor.*—

*Gross Description:* The tumor was somewhat grayish in color and was extremely hard. It weighed 525 gm. It measured  $15\frac{1}{2}$  cm. in length; its width varied from 4 cm. to 8 cm. It seemed to consist of two lobes, having the general contour of a thymus gland, and the lower end presented a notch. The cut surface had a white glistening appearance.

*Microscopic Description:* Histologic examination of the tumor showed a dense fibrous stroma in which were embedded large numbers of epithelial cells and a small number of lymphoblasts. The epithelial cells occurred singly and in small nests. In some areas there was a tendency to alveolar arrangement of the cells. Most of the epithelial cells were cuboidal or cylindrical. The nuclei were large and vesicular and showed many mitotic figures. The cytoplasm was pale.

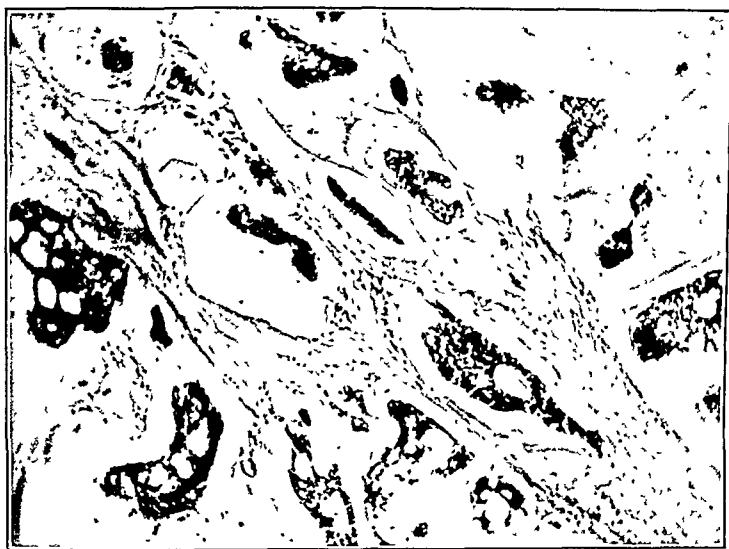


Fig. 4.—Microscopic appearance of tumor showing tendency to take on alveolar arrangement.

COMMENT

This tumor presents the outstanding characteristics which are used as criteria for the diagnosis of primary carcinoma of the thymus gland. These are as follows:

1. *Location:* That normally occupied by the thymus gland.
2. *Shape:* The contour of the thymus gland is simulated and there is a notch at the lower end.
3. *Structure:* The histologic structure is that of an epitheliomatous growth in a dense fibrous stroma. Lymphoid tissue is also present.
4. There is no evidence of any primary tumor elsewhere in the body which may have metastasized to the thymus.

Most thymic tumors have the above characteristics, with the exception that the histologic structure varies with the origin of the tumor.

## SUMMARY

- 1 A case of primary carcinoma of the thymus gland is presented
- 2 Dyspnea, cyanosis, swelling of the neck and face, and exophthalmos were the outstanding symptoms
- 3 The first symptoms were noted less than three months before the patient died
- 4 The tumor was not radiosensitive
- 5 A typical carcinomatous structure was found upon histologic examination

## REFERENCES

- 1 Crosby, E H Malignant Tumors of Thymus Gland, *Am J Cancer* 16 461, 1932
- 2 Symmers, D Malignant Tumors and Tumor Like Growths of Thymic Region, *Ann Surg* 95 544, 1932
- 3 McDonald, S, Jr Case of Reticulum Cell Carcinoma of Thymus, *J Path & Bact* 35 1, 1932
- 4 Cesaris Demel, V, Jr Di un caso di cancro alveolare del timo, *Pathologica* 24 607, 1932
- 5 Touroff, A S W Transitional Cell Carcinoma of Thymus in a Child, *J Mt Sinai Hosp* 1 17, 1934
- 6 Collins, J J Case of Carcinoma of Thymus *Radiology* 18 1148, 1932
- 7 Ewing, James Neoplastic Diseases, Philadelphia, 1919, W B Saunders Co, p 889
- 8 Leyton, O, Turnbull, H M, and Bratton, A B Primary Cancer of Thymus With Pluriglandular Disturbances, *J Path & Bact* 34 635, 1931

## \* FEVER ACCOMPANYING THE INDUCED RETICULOCYTE CRISIS OF PERNICIOUS ANEMIA\*

W M FOWLER, M D, IOWA CITY, IA

IN A series of 206 cases of pernicious anemia in which remissions were induced by liver therapy we have observed fever, ranging from 102.4° F to 106° F, coincident with the reticulocyte crisis in 8 cases. In each of these 8 cases a concentrated form of liver extract was administered, to one patient intramuscularly, to the others orally. Every conceivable extraneous cause for fever, such as intercurrent infection and signs of inflammation at the site of injection, was rigidly excluded. In each case the temperature rose rather suddenly, returning to normal more gradually over a period of twenty four to forty-eight hours. In 5 of the 8 cases reticulocyte counts were being done daily. In 2 of these, the highest temperature coincided exactly with the highest reticulocyte count, in the other 3, it occurred during the period of decline. In the 3 cases in which reticulocyte counts were not being made, the precise relationship between temperature and reticulocytosis was not ascertained, but in each the fever appeared during the time when the crisis was to be expected. The patient whose temperature reached 106° F had a coincident reticulocytosis of 45 per cent, he was receiving extractum by mouth in doses of 18 capsules per day. In several of the cases, the fever was accompanied by a slight chill, in the others, there were no associated symptoms of any kind. None of the patients had a leucocytosis

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TABLE I

	AGE	SEX	R. B. C. MILLIONS	HB PER CENT	RETICULO- CYTES		TEMPERATURE		THERAPY	TEMPERA- TURE BEFORE THERAPY
					HEIGHT	DAY	HEIGHT	DAY		
S. H.	58	F	2.25	87	----	--	102.4	8	Liver extract	100° R
J. H.	60	M	0.96	20	----	--	103.0	10	Liver extract	Normal
S. H.	56	F	0.70	25	26.8%	7	102.2	9	Liver extract	99°
G. W.	63	M	2.38	65	----	--	105.0	14	Ventriculin	Normal
O. F.	63	M	2.36	63	13.8%	7	102.4	14	Liver extract	100°
									Liver extract	
J. E.	60	M	1.65	40	20.0%	7	102.0	7	I. M.	100°
E. W.	62	M	0.66	18	28.0%	6	105.0	10	Liver extract	101°
O. J.	60	M	0.85	11	45.0%	8	106.0	8	Extralin	100°

There was no apparent relationship between the occurrence of fever and the pre-therapeutic erythrocyte level. The continuous low-grade fever characteristic of pernicious anemia during relapse which was manifested by many of these 206 patients disappeared in most cases after the reticulocyte crisis.

We have searched the literature without finding any allusion to fever with reticulocytosis, which in itself is strong evidence that the fever we observed was not referable to the reticulocytosis alone, and we are unable to give a satisfactory explanation of the mechanism. It has been shown that reticulocytes are more fragile than normal erythrocytes and that they do not mature to a significant extent in the circulating blood.<sup>1</sup> The fact that the cholemia is subsiding at the time when the reticulocyte crisis occurs excludes the possibility that increased hemolysis may be the causative factor. Reticulocytes consume more oxygen than mature cells,<sup>2</sup> but inasmuch as there is a gradual decrease in the basal metabolic rate after liver therapy is begun<sup>3</sup> hypermetabolism as a basis for the fever is not tenable. If a foreign protein reaction or an allergic reaction to decomposition products of the erythrocytes or their nuclei is responsible, it is necessary to assume an individual susceptibility in order to account for the infrequent occurrence of the fever.

## REFERENCES

1. Mermod, C., and Dock, W.: Fragility and Maturation of Reticulocytes, *Arch. Int. Med.* 55: 52, 1935.
2. Harrop, G. A., Jr.: The Oxygen Consumption of Human Erythrocytes, *Arch. Int. Med.* 23: 745, 1919.
3. Balbridge, C. W., and Barer, A. P.: Studies on the Relationship Between Oxygen Consumption and Nitrogen Metabolism. I. In Pernicious Anemia, *J. Clin. Investigation* 10: 529, 1931.

# THE BLOOD PICTURE IN TWO CASES OF AGRANULOCYTOSIS\*

## FOLLOWING TREATMENT WITH NEOAPSPHENAMINE WITH SPECIAL REFERENCE TO MYELOCYTES AND JUVENILES

J P CRAWFORD MAJ M C U S ARMY WASHINGTON, D C

RECENT literature contains numerous articles on agranulocytosis and like conditions. In but a few instances have there been noted any increase in the myelocytes and juveniles in the circulating blood. Roberts and Kracke,<sup>1</sup> Kerlin,<sup>2</sup> Bethell,<sup>3</sup> Jackson,<sup>4</sup> Schilling. Jackson states "During convalescence from agranulocytosis there may be, and usually is an outpouring of myelocytes but this stage is soon passed and clinical improvement is evident coincidentally."

It is possible that we have been relying too much on the technician who may or may not be qualified to make accurate differentiation of the various white blood cells, especially those forms which are not encountered in most daily routine examinations. Or perhaps some qualified individual may become interested, make a few white blood counts and then turn the work over to the technician, thus missing the true picture.

The oxydase reaction in doubtful cases is a valuable aid. The method, as given by Graham,<sup>5</sup> is simple and was used as a check on the Wright's stain in the two cases reported in this paper. During the period where there were no segmented and staff forms present the differential count of the granular and nongranular white blood cells was almost identical when the oxydase reaction was compared with slides stained by Wright's method. The myelocytes and juveniles gave a very clear picture with red brown granules in the cytoplasm when stained by the Graham method.

Kracke<sup>1</sup> states "The term agranulocytosis means an increase in the number of immature granular cells, whereas granulocytopenia expresses a decrease of granular cells." These two cases developed an increase in the number of immature granular cells. At the same time there was a marked decrease or absence of adult segmented and staff forms.

The following is the history and findings of these two patients. (The medical treatment of the cases for the agranulocytosis was under the supervision of Major H C Dooling, Medical Corps, U S Army.)

### CASE REPORT

CASE 1—(Table I and Chart 1) An adult white male, aged twenty seven years, weight 140 pounds, well developed and nourished.

His previous history showed that he was treated for gonorrhea in the early part of October 1934. On the twenty ninth of the same month he developed a chancre on his penis, the dark field being positive for *Treponema pallidum*. Antisyphilitic treatment was begun on

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the same day, consisting of 0.1 gm. bismuth salicylate intramuscularly and 0.6 gm. neoarsphenamine intravenously. One week later a second treatment was given, increasing the neoarsphenamine to 0.9 gm. Two days after the second injection there was noticed a macular eruption scattered diffusely over the entire body. Three days later the eruption was of a deeper color with some itching, and the conjunctiva was congested.

At this time his blood chemistry was within normal limits. The icteric index was 3. The direct and indirect Van den Bergh tests were negative. The white blood count was 5,600; differential 61 per cent segmented, 4 per cent staff, 8 per cent juvenile, 6 per cent eosinophiles, 12 per cent lymphocytes, and 9 per cent monocytes.

By November 12, the patient being apparently well, it was decided to continue the antisyphilitic therapy. Bismuth 0.1 gm. and neoarsphenamine 0.5 gm. were given. There was no evidence of any reaction and seven days later the same treatment was repeated. This made a total of 1 gm. neoarsphenamine in eight days.

Five days after the last treatment he was readmitted to the hospital with a diagnosis of external hemorrhoids. He had been doing duty for the past twelve days.

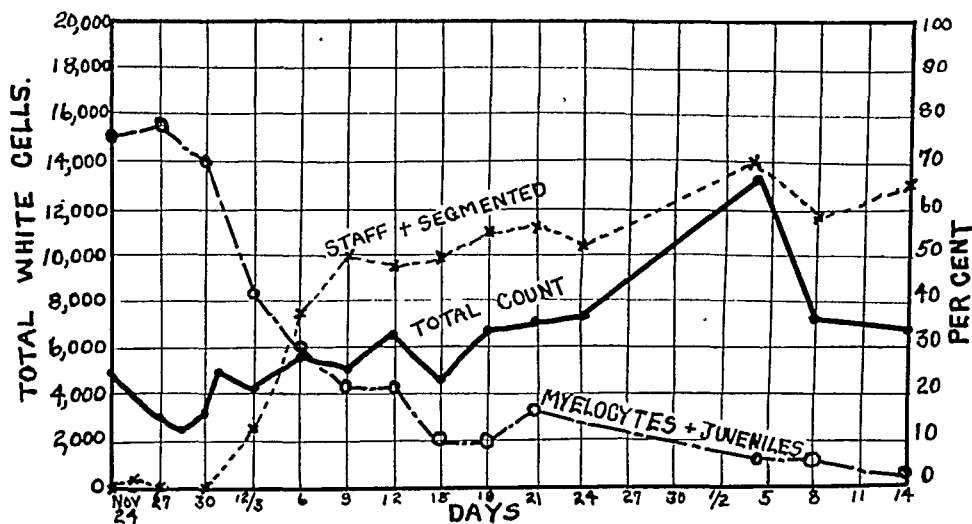


Chart 1.

Physical examination on admission was essentially negative, except for a slight reddening of the throat and some hypertrophy of the tonsils. There were no ulcerations on the tonsils or gums. There were three small external hemorrhoids present.

On admission the temperature was 97.4° F., pulse 128, and respiration 20. At 8 P.M. the temperature was 102° F. The following day he complained of marked weakness and seemed acutely ill. His temperature was 104°. Upon reexamination, the only positive findings were the congested pharynx and the external hemorrhoids. A blood count revealed a total of 4,900 white cells. There were no segmented cells present, but there were a considerable number of myelocytes and juveniles. The patient was transferred to the Medical Service with a diagnosis of agranulocytosis following antisyphilitic treatment with neoarsphenamine.

The treatment consisted of nucleotide (K-96) and glucose intravenously. The nucleotide was given for eight consecutive days. Liver extract was started on the second day, both intramuscularly and orally. He continued to have a high temperature for nine days. During this time he seemed prostrated and the gums and mucous membrane of the pharynx became markedly ulcerated. The breath was very foul. The hemorrhoids ulcerated and disappeared as if they had been burned off with a cautery.

The patch test with neoarsphenamine was negative on two different days. The fragility test revealed a slight increase in the resistance of the red blood cells, hemolysis beginning in 0.42 and complete in 0.30 per cent saline.

The clinical course closely paralleled the change in the blood picture, the patient improving markedly as the segmented and staff forms returned to the circulating blood. This case was more severe than Case 2 and it is interesting to note that there were a greater number of myelocytes and juveniles and less eosinophiles and basophiles than in the second case. Also he did not show as marked an increase in the segmented forms during recovery as Case 2. It was also noted that the staff forms appeared before the segmented. There was only one staff and no segmented forms noted during the first seven days.

TABLE I

## CASE 1 TOTAL WHITE BLOOD CELLS AND DIFFERENTIAL COUNT

DATE	TOTAL COUNT	BASO PHILES	EOSINO PHILES	MYELO CYTES	JUVE NILE	STAFF	SEG MENTED	LYMPH OCYTES	MONO CYTES	MISCEL LANE OUS
11/24/34	4,900		2	55	21			15	3	1
25	4,200			52	26	1		18	3	
26	3,500	1		47	25			14	13	
27	3,100			72	5			16	7	
28	2,500		1	53	14			24	4	4
29	2,950	1	1	31	29			33	5	
30	3,000	2	2	34	36			20	6	
12/ 1/34	4,700	1	4	23	29	2	1	37	3	
2	4,350	1	6	16	32	9	1	29	6	
3	4,100	1	11	17	24	9	4	31	3	
4	5,800		4	17	22	13	8	28	8	
5	5,700	1	1	21	15	14	24	22	2	
7	6,500			6	23	9	33	29	1	
9	5,500		2	7	15	10	40	24	2	
11	5,300		2	5	12	11	37	25	8	
12	6,400		3	9	12	5	42	24	5	
13	7,400	1	5	4	12	2	47	20	4	
14	6,550	1	2	3	9	3	38	39	6	
15	4,550		5	2	8	3	45	30	7	
17	5,350		2	6	9	5	49	22	7	
19	6,600		3		10	4	52	23	6	
22	6,950	1	6		17	4	46	23	1	
26	7,100		4		9	1	50	32	4	
1/ 4/35	12,750		1		6	4	66	22	1	
8	7,350	1	4		6	4	55	28	2	
15	7,000			1	4	5	67	20	3	
19	7,800			1	2		65	29	2	
22	8,600		3	1	7	9	61	11	8	
2/14/35	9,950	1	2		6	3	49	34	5	

In the graphic Chart "1" the myelocytes and juveniles and the staff and segmented are grouped together. The clinical course of the patient corresponded with this grouping and as the staff and segmented increased and the myelocytes and juveniles decreased, the condition of the patient improved accordingly.

CASE 2—(Table II and Chart 2) Patient 2 was a white male, aged nineteen, weight 160, a soldier, well developed and nourished. He had bronchopneumonia ten years ago and the usual diseases of childhood. He contracted gonorrhea in April, 1934, and was admitted to the hospital in October, 1934, for chronic urethritis. It was then found that his Wassermann and Kahn tests, repeatedly, were strongly positive. He was therefore started on antisyphilitic treatment as follows: Oct 14, 1934, 0.1 gm bismuth subglycolate and 0.5 gm of neoarsphenamine. October 21, the same amount of bismuth with 0.9 gm of neoarsphenamine. November 3 this was again repeated. This made a total of 2.3 gm of neoarsphenamine over a period of twenty one days. There were no reactions following the first two treatments. However, from the time of the last treatment he had been feeling weak, having a mild sore throat. He had

the same day, consisting of 0.1 gm. bismuth salicylate intramuscularly and 0.6 gm. neoarsphenamine intravenously. One week later a second treatment was given, increasing the neoarsphenamine to 0.9 gm. Two days after the second injection there was noticed a macular eruption scattered diffusely over the entire body. Three days later the eruption was of a deeper color with some itching, and the conjunctiva was congested.

At this time his blood chemistry was within normal limits. The icteric index was 3. The direct and indirect Van den Bergh tests were negative. The white blood count was 5,600; differential 61 per cent segmented, 4 per cent staff, 8 per cent juvenile, 6 per cent eosinophiles, 12 per cent lymphocytes, and 9 per cent monocytes.

By November 12, the patient being apparently well, it was decided to continue the antisyphilitic therapy. Bismuth 0.1 gm. and neoarsphenamine 0.5 gm. were given. There was no evidence of any reaction and seven days later the same treatment was repeated. This made a total of 1 gm. neoarsphenamine in eight days.

Five days after the last treatment he was readmitted to the hospital with a diagnosis of external hemorrhoids. He had been doing duty for the past twelve days.

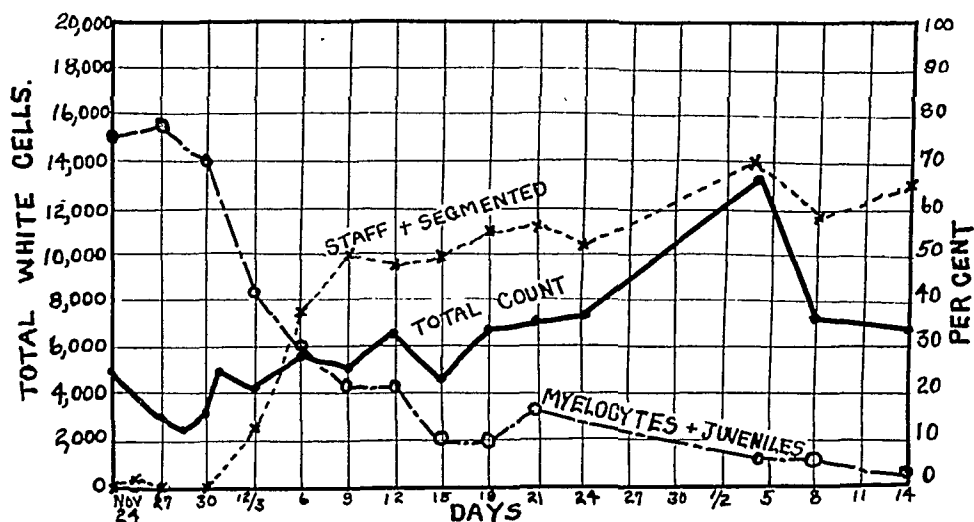


Chart 1.

Physical examination on admission was essentially negative, except for a slight reddening of the throat and some hypertrophy of the tonsils. There were no ulcerations on the tonsils or gums. There were three small external hemorrhoids present.

On admission the temperature was 97.4° F., pulse 128, and respiration 20. At 8 P.M. the temperature was 102° F. The following day he complained of marked weakness and seemed acutely ill. His temperature was 104°. Upon reexamination, the only positive findings were the congested pharynx and the external hemorrhoids. A blood count revealed a total of 4,900 white cells. There were no segmented cells present, but there were a considerable number of myelocytes and juveniles. The patient was transferred to the Medical Service with a diagnosis of agranulocytosis following antisyphilitic treatment with neoarsphenamine.

The treatment consisted of nucleotide (K-96) and glucose intravenously. The nucleotide was given for eight consecutive days. Liver extract was started on the second day, both intramuscularly and orally. He continued to have a high temperature for nine days. During this time he seemed prostrated and the gums and mucous membrane of the pharynx became markedly ulcerated. The breath was very foul. The hemorrhoids ulcerated and disappeared as if they had been burned off with a cautery.

Other laboratory findings were throat culture positive for hemolytic streptococci and negative for Vincent's organisms during the acute stage. A blood culture taken November 10 was negative. The icteric index November 13 was 3.75. A patch test with neoviraphenamine was markedly positive November 13. However, this test repeated after his recovery, January 20, was negative. The urine was within the normal limits. The fragility test showed a slight increased fragility of the red blood cells, hemolysis beginning in 0.48 and complete in 0.34 per cent saline. Many different bacteria were found in smears taken from the ulcers during the period November 10 to 12, yet no white blood cells were present.

Table II shows the progress of the blood count with special reference to the differential white cell count. It shows a marked shift to the left (Schilling index) with a later shift to the right as the patient improved. During convalescence there was a marked hyperleucocytosis involving the segmented, staff, and juvenile forms, the highest count being about eight days after his first signs of improvement.

Chart 2 gives a grouping of the segmented and staff and the myelocytes together, charted with the total white cell count.

TABLE II

CASE 2

NUCLEOTIDE	TEMPERATURE	DATE	TOTAL COUNT	EOSINOPHILS	MYELOCYTES	JUVENILE	STAFF	SEGMENTED	LYMPHOCYTES	MONOCYTES	MISCELLANEOUS
10 cc	104.0	11/9	3,800	4	12	8	3	12	19	17	12
10 cc	104.0	11/10	2,700	1	1	10	57	3	16	5	12
10 cc	102.4	11/11	4,100			6	45	3	21	18	12
10 cc	102.2	11/12	4,600	1	2	2	22	8	34	13	12
	101.0	11/13	5,250		2		8	18	28	31	4
	99.4	11/14	6,400			1	6	13	57	18	5
	99.6	11/15	7,800		3	1	13	12	52	14	3
	99.6	11/16	10,600		2	1	6	10	61	20	2
	99.0	11/19	13,400			1	5	6	68	18	6
	98.6	11/20	16,150	1			5	6	71	11	3
	99.0	11/21	14,500	1			5	9	72	9	1
	98.0	11/30	13,800			2	1	4	83	9	2
	99.0	12/3	9,400				3	1	82	12	2
	99.0	12/7	17,200				2	4	77	9	8
	98.0	12/9	9,200			1	3	4	70	19	3
	99.0	12/14	11,600			2	7	2	73	9	7
	99.8	12/17	13,600	1		3	10	4	61	19	2
	99.0	12/26	9,950	2			9	1	71	12	5
	98.0	1/4	6,500	4			3	6	66	14	7
	99.0	1/9	9,400	4			5	1	61	26	4
	99.0	2/16	14,400	1	2		2	1	80	11	3

NOTE. Under miscellaneous were noted lymphoblasts and basket cells. Blood platelets decreased in number about one half for first four days, platelets being markedly increased in size many as large as red blood cells. From November 13 on the platelets were markedly increased in number and showed marked variations in size.

The red blood count was normal throughout being from 4,500,000 to 5,000,000. The eosinophiles and basophiles present during the first five days were of the juvenile type.

## COMMENT

It will be noted that these patients came to the hospital without ulcerative lesions and developed them later. One patient was admitted with external hemorrhoids that ulcerated and completely sloughed about the sixth day after admission. Therefore, it seems probable that the ulcers on the

been doing his regular duty, consisting of the rather strenuous cavalry drill. On the morning of November 9, while at drill, this being six days after his last intravenous neocarsphenamine, he became dizzy and dismounted from his horse. He had a few chills, vomited, and then developed a severe headache, together with a pain in his chest.

On admission to the hospital he was acutely ill. His skin was flushed, his respirations were 30, temperature  $101^{\circ}$  F., and pulse 120, being full and regular. He was semilethargic, and could be aroused with difficulty to answer questions. He was slightly irrational when aroused. There was a foul odor to his breath and his throat was red. The gums were of normal appearance. The tonsils were moderately inflamed, but there were no ulcers present. No enlarged glands were noted. The heart sounds were clear, and there were no murmurs present. There was a slight dullness at the right base with decreased breath sounds in this region. The abdomen was scaphoid. The liver and spleen were not palpable. He was there fore put under observation for pneumonia.

The x ray revealed only a residual of an old pneumonia, and compared with the films taken on his release, two years ago, there were less markings. There was no evidence of any acute process in the lungs. However, a blood count was made and the total white count was

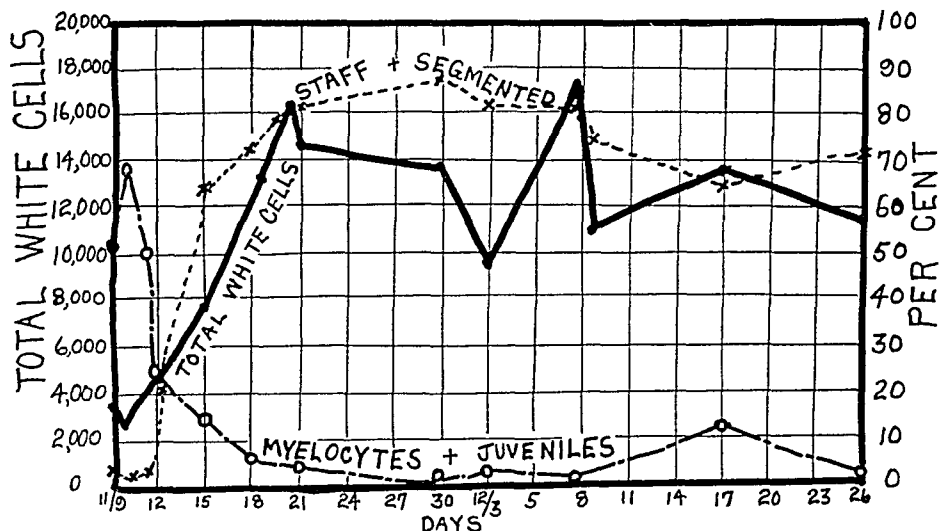


Chart 2.

3,800 with only two segmented cells per hundred noted. There were a considerable number of juveniles and myelocytes present.

The next day, November 10, his condition was grave. His throat was red, yet there were no ulcerations on the gums or tonsils. He was given 10 c.c. nucleotide (K-96) intramuscularly. He was nauseated during the entire day and could not retain his food. Thirty cubic centimeters of 50 per cent glucose was given intravenously. On this day, not a single segmented cell could be found on the slide. The total count was 2,700 and 10 myelocytes and 57 juveniles per 100 were noted.

The following day, November 11, the same treatment was repeated with the addition of an antiseptic gargle and mouth wash, as he was developing a slight ulceration on the gum margin. He was retaining some food. One segmented per hundred white cells was noted.

The fourth day, November 12, the same treatment was continued with the addition of liver. The ulcerations on the gums had increased. The temperature which had been  $101^{\circ}$  on the first two days was now  $102.2^{\circ}$  and there appeared 13 per cent segmented forms in the blood smear. From this time on he made a rapid recovery, the improvement paralleled the increase in the segmented and staff forms in his blood smears.

The temperature continued to show a slight afternoon elevation during his stay in the hospital.

There are cases where it seems very probable that the toxic products of bacteria may cause dysfunction of the bone marrow. However, one must consider that the bone marrow may be abnormal from the start from various causes. Perhaps allergy in the form of sensitivity to certain drugs may play an important part.

The following case is an example.

A white male, aged sixty-two years, was admitted to the hospital two days after going to a chiropodist who pared a corn from his foot. When admitted he was seriously ill. His temperature was 105° F and his pulse rapid. The blood counts revealed 500 to 1,000 white blood cells, all of which were small lymphocytes. A blood culture was positive for hemolytic streptococci. He died four days after the chiropodist attended him. Death in this case was probably due to septicemia, as four days with no phagocytes present in the blood stream and no other complications would be the exception. However, a poor functioning bone marrow may have been the basic cause.

A person who patronized the chiropodist might also patronize the corner drug store and use drugs that might be dangerous. Certain drugs are known to affect the bone marrow and with a loss of the phagocytes, bacteria may invade any part of the body. The history as to the use of drugs was not obtained in this case as little was known at that time with regard to these harmful drugs. Now that it is a well established fact, an attempt to elicit the history of drug administration should be made in these cases.

Patients 1 and 2 were probably allergic to neoarsphenamine. One gave a marked positive patch test. The other developed an allergic type of reaction.

Another patient was observed by me two years previously. He was sensitive to neoarsphenamine and developed a reaction every time it was given. It was six months before his blood picture returned to normal. He was also sensitive to quinine. This patient was given quinine to determine what effect it might have on his blood picture. Roth<sup>8</sup> states that quinine causes a pronounced leucocytosis, at first there is an increase in the number of lymphocytes which he believes is due to contraction of the spleen and lymphatic tissue, but later there is an increase in the number of polymorphonuclear cells. Quinine in this case, in doses of 5 gr. three times daily, was given over a period of six days. There was an increase in the segmented cells during this period. However, as this man was sensitive to quinine as well as neoarsphenamine, this treatment was discontinued.

#### CONCLUSIONS

Two cases are presented, following treatment with neoarsphenamine, with an increase in the circulating myelocytes and juveniles and a decrease in the number of segmented and (stab) staff forms with a marked shift to the left in the Schilling index.

1. As these patients were admitted with no ulcerative lesions and later there appeared a disintegration of the tissue where pathogenic bacteria are in intimate contact with the mucous membranes, namely, the gums, tonsils, and anus, it is presumed that these lesions were due to the absence of the phagocytes (the segmented and staff forms) from the circulating blood.

2. Smears taken from the lesions revealed only bacteria and débris, there being no white blood cells present. Therefore, the bacteria flourished during the absence of the phagocytes.

3. Myelocytes and juveniles apparently have no protective value in combating local infections, there being a considerable number of these forms in the circulating blood before and during the time that the ulcerations appeared and progressed.

4. The segmented and staff forms, by means of their flexibility, may pass through the capillary walls and act as phagocytes, whereas, the myelocytes and juveniles with their large single nuclei, were unable to pass between the intercellular spaces.

5. From the standpoint of the clinician, the granulocyte count made according to Schilling, might be divided into two main groups, i.e., those cells possessing phagocytic powers (staff and segmented), and those having little if any phagocytic powers (myelocytes and juveniles).

6. With this grouping it was noted that the clinical picture of the patient corresponded with the charts. That is, there was noted a marked improvement in the patient when the staff and segmented forms appeared in the circulating blood and the myelocytes and juveniles decreased in numbers.

7. It is not probable that the nucleotide was the factor causing the myelocytes and juveniles to appear in the circulating blood, as these forms were present in increased numbers before the nucleotide was given.

8. The neoarsphenamine was the cause of the condition by its effect on the bone marrow.

9. These patients were probably allergic to neoarsphenamine.

#### REFERENCES

1. Roberts, S. R., and Kracke, R. R.: Agranulocytosis, *J. A. M. A.* 95: 780, 1930.
2. Kerlin, W. S.: Differential Diagnosis Between Agranulocytic Angina and Acute Leukemia, *New Orleans M. & S. J.* 87: 759, 1935.
3. Bethell, F. H.: The Response to Infection in Bone Marrow Dyscrasias, *J. LAB. & CLIN. MED.* 20: 362, 1935.
4. Jackson, H.: The Differential Diagnosis of Agranulocytic Angina From Acute Leukemia, *Am. J. M. Sc.* 188: 604, 1934.
5. Schilling, V.: *Das Blutbild und seine klinische Verwertung*, Fischer, Jena, 1926; translated by R. B. H. Gradwohl, St. Louis, 1929, The C. V. Mosby Co.
6. Graham, G. S.: Benzidine as a Peroxidase Reagent for Blood Smears and Tissues, *J. Med. Res.* 34: 15, 1918.
7. Kracke, R. R.: A Review of Granulocytopenia (Agranulocytosis), *J. LAB. & CLIN. MED.* 17: 993, 1932.
8. Roth, G. B.: The Action of Quinine on Leucocytes, *J. Pharmacol. & Exper. Therap.* 3: 470, 1911-12.

# STUDIES ON THE CIRCULATION THE DYE INJECTION METHOD\*

## THE EFFECT OF DIGITALIS UPON PATIENTS WITH NORMAL CARDIOVASCULAR SYSTEMS

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DIGITALIS has been known for many years as the sovereign remedy for heart disease. The mechanism of its action on the circulatory system in health and disease has been the subject of numerous investigations. In simple perfusion experiments the effect of digitalis upon the isolated frog or mammalian heart is that the organ empties itself more completely. In the more intricate experiments as the heart-lung preparation in the dog, Cohn and Steele<sup>1</sup> have shown that the administration of therapeutic doses of digitalis to the failing and dilated heart causes an increase in minute output, whereas in the case of the healthy heart, normal in size, the opposite is true. They felt that the effect on the tone of the heart is the outstanding phenomenon. They refer to tone as meaning the diastolic volume of the heart, the length of the fibers in a given case or the fitness of the muscle fibers.

Abundant evidence has been forthcoming in recent years to show that the whole story of the action of digitalis in the body cannot be adduced from experiments upon isolated organs, but rather upon the interpretation of the changes that occur in the intact organism after readjustments have taken place in the body as a whole.

In the normal dog, Harrison and Leonard,<sup>2</sup> Stewart and Cohn,<sup>3</sup> and Dock and Tainter<sup>4</sup> found a decrease in output following the administration of digitalis. Stewart and Cohn are of the opinion that the drug acts primarily upon the heart and that the decrease in output is the consequence of the heart becoming small, whereas Dock and Tainter express the opinion that the decrease in output is the result of the constricting action of the drug upon the hepatic veins. They believe that in the dog, the constriction is at the caval openings of the veins, whereas in man and cat, the constriction occurs in the hepatic vein radicals. As proof of their contention they cite experiments which show a fall in right auricular pressure and a simultaneous rise in portal vein pressure and liver engorgement following the administration of digitalis.

It would appear, therefore, in the light of the above conclusions, that the pharmacologic action of digitalis in the body of the dog is still open to further investigation.

In the normal human being the studies of the action of digitalis have been limited principally to the cardiac size and output, venous pressure, velocity of blood flow and total blood volume. Following the administration of this drug, Burwell and others,<sup>5</sup> using the carbon dioxide method, and

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**Results.**—Table 1 shows a summary of the findings. With the exception of the pulse and the blood pressure each value in every case has a column showing the percentage of the initial normal, and Fig. 1 is constructed to show the median, as well as the case distribution of each factor in terms of percentage of the initial normal. It will be seen in glancing at Fig. 1 that some of the factors show a rather decided deviation from the normal median values; at the same time the scatter of the individual values is quite prominent.

**Effect on Venous Pressure.**—The median venous pressure of the group following digitalis therapy is decidedly decreased, being 61 per cent of normal. The changes range from 20 to 150 per cent of the initial normal.

The cases which show no change or a decrease in venous pressure likewise show no change or a decrease in the flow. Case 130 is an exception. Case 130 shows a marked increase in venous pressure, as well as a marked increase

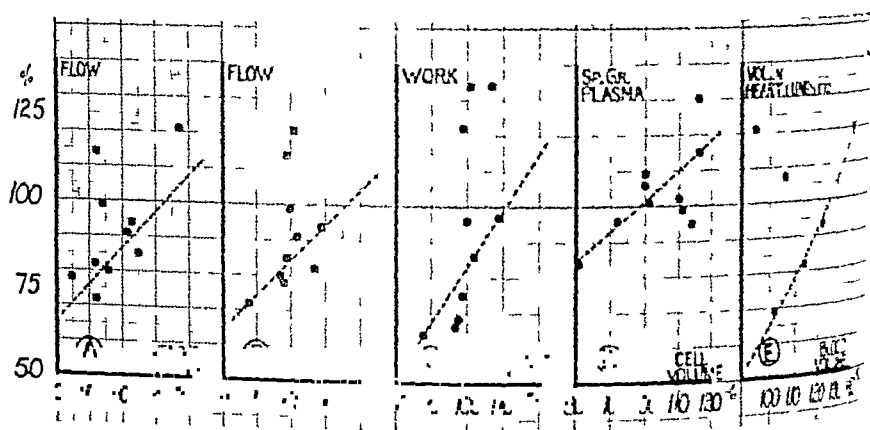


Fig. 2—Showing the interrelationship of the various factors in terms of per cent of initial normal. Flow, Output per minute. Work, Flow per minute times mean blood flow. Heart size, Cardiac silhouette area. Sp. gr. Pl., Specific gravity of plasma. Vol. in heart and lungs, Volume of circulating blood between point of injection (antecubital) and point of sampling (femoral artery).

flow. However, in the individual cases of the groups when the venous pressure and flow are plotted against each other there exists a close linear relationship in not more than 70 per cent of the cases (Fig. 2 A).

There is no correlation between venous pressure and the other factors studied, namely, the velocity of blood flow, the stroke volume, the amount of blood in heart and lungs, the total blood volume, the heart area, the specific gravity of the plasma, or the work.

**Velocity of Blood Flow.**—While there is a tendency to increase in the velocity of blood flow, nevertheless the changes are not significant; there is no correlation with the other factors.

**Flow.**—The median flow is 88 per cent of initial normal. The changes are from 72 to 121 per cent of initial normal.

In 80 per cent of the cases there is a definite correlation between flow and heart area (Fig. 2 B).

TABLE I\*  
 TWENTY FOUR HOUR EFFECT OF DIGITALIS (1 TO 1.2 GM) IN THE HUMAN WITH NORMAL CARDIOVASCULAR SYSTEM

CASE	AGE	ULSE	B P	V P	% INITIAL	A T	% INITIAL	LOW/SA	% INITIAL	V/SA	% INITIAL	BF/SA	% INITIAL	WORK/SA	% INITIAL	HEART SIZE	% INITIAL	CELL VOL	% INITIAL	SP GP	% INITIAL
166	23	72	110/70	70	93	90	121	3.48	94	1.19	107	2.68	106	4265	99	99	520	520	P 10268	1090	
161	19	87	124/80	40	92	109	121	3.28	94	1.28	107	2.75	106	4150	97	108	520	449	P 10279	1090	
161	19	87	124/80	40	92	92	76	4.23	121	1.35	82	2.41	114	5875	104	104	449	449	P 10284	1090	
164	18	72	140/84	60	150	70	76	5.14	121	1.11	82	2.75	114	7830	134	103	455	455	P 10261	1005	
164	18	72	120/75	105	148	148	89	3.81	99	1.97	69	2.53	102	5080	112	112	455	455	P 10248	1005	
164	27	81	110/80	60	97	132	89	3.77	99	1.37	69	2.59	102	4875	96	112	326	326	P 10273	1300	
160	27	81	116/70	83	92	92	89	3.32	82	1.35	116	1.89	140	2622	106	106	385	385	P 10228	1064	
160	39	71	120/70	40	48	140	152	2.72	82	1.56	116	1.89	140	4538	113	113	385	385	P 10279	1064	
160	39	71	110/62	82	131	131	95	3.88	114	1.53	120	2.65	140	3513	134	116	405	405	P 10243	1153	
161	45	67	120/65	40	49	125	95	4.43	114	1.83	120	2.65	140	6530	122	124	350	350	P 10227	1153	
161	45	67	160/80	30	260	76	78	4.00	72	1.33	72	2.79	140	4200	64	109	474	474	P 10266	994	
163	30	81	128/80	55	50	204	78	5.47	72	2.45	72	2.79	140	4200	64	109	474	474	P 10237	994	
165	29	75	106/70	35	64	123	162	4.76	80	1.30	113	2.18	129	5220	67	117	520	520	P 10202	1020	
145	29	75	115/84	75	125	125	124	2.72	78	1.20	100	2.18	129	3670	123	123	493	493	P 10254	950	
145	29	75	115/84	75	125	125	124	2.72	78	1.20	100	2.18	129	3670	123	123	493	493	P 10254	950	
142	40	83	100/75	15	20	153	124	2.12	78	1.19	100	2.18	129	2540	69	120	363	363	P 10271	836	
142	40	83	125/90	30	136	136	136	3.90	85	1.48	93	2.36	129	5680	112	112	468	468	P 10271	836	
134	38	63	110/80	30	100	146	107	3.30	85	1.38	93	2.36	129	4263	75	111	99	99	P 10271	836	
134	38	88	128/95	80	75	75	75	3.65	85	0.78	93	2.05	129	5510	90	90	480	480	P 10271	836	
134	38	69	124/85	70	87	84	112	3.33	91	0.94	121	1.95	95	4712	86	92	442	442	P 10270	961	

\* V P Venous pressure A T rate of flow between antecubital vein and femoral artery

Flow output per minute V volume of circulating blood l between point of infection (antecubital vein) and point of sampling (femoral artery) B V total circulating blood l volume S A surface area

Work flow per minute times mean blood pressure Heart Size cardiac silhouette area Sp P l specific gravity of plasma

\*V P Venous pressure A T rate of flow between antecubital vein and femoral artery Flow output per minute V volume of circulating blood between point of injection (antecubital vein) and point of sampling (femoral artery) B V total circulating blood volume S A surface area Work flow per minute times mean blood pressure Heart Size cardiac silhouette area Sp Gr Pl specific gravity of plasma

*Effect on Stroke Volume.*—The median stroke volume is increased to 110 per cent of initial normal. The deviations are from 67 to 159 per cent of initial normal. There is no correlation with the other factors.

*Total Blood Volume.*—Only five cases were determined in this series. There is a tendency for the total circulating blood volume to be increased. This is not in keeping with the findings reported by Wollheim; nevertheless, the conditions are hardly comparable, in that he injected the digitalis and made his determinations soon after the injection (twenty minutes), whereas with us the drug was given by mouth, and the determination was made twenty-four hours later.

In four of the cases there is a striking linear relationship between the total blood volume and the volume in the heart and lungs (the circulating blood between the point of injection [antecubital vein] and the corresponding vessels to the point of sampling [femoral artery]). The latter we have termed *V* (Fig. 2 E). The *V* in four of these cases is decreased in about the same proportion as the circulating blood volume in the greater circulation is increased. It is evident that the engorgement is either in the extremities or in the splanchnic area.

*Heart Area and Work.*—The percentage of deviation from initial normal in each of these two factors has a scatter which causes the median value of the heart area to fall near the initial normal, whereas the median for work falls well below. However, when the values are plotted against each other, we find that seven out of the ten cases show some linear correlation (Fig. 2 C). There is no correlation of the other factors with work.

*Cell Volume and Specific Gravity of the Plasma.*—The median values of these two factors show little or no change following the administration of digitalis (Fig. 1). The scatter of the individual cases in the cell volume is from 81 to 116 per cent of initial normal, and in the specific gravity of the plasma from 84 to 116 per cent of initial normal. There is some correlation between the cell volume and the specific gravity of the plasma (Fig. 2 D). No such correlation exists with the other factors studied.

#### COMMENT

In reviewing the literature of the action of digitalis on the human being with normal cardiovascular system, one is impressed with the decided divergence of opinion by some of the most outstanding workers in this field. We find that one school of thought stresses the action of the drug upon the heart, whereas another school emphasizes its action upon the peripheral circulation. The methods we have used in the study of hemodynamics have the advantage of determining almost simultaneously without change in the position of the patient, the venous pressure, vital capacity, velocity of blood flow, flow, total blood volume, etc.

Our results show that twenty-four hours after the oral administration of fairly large doses of digitalis, the heart area may increase, decrease or remain unchanged. The flow on the whole is diminished, though some cases show an increase and others are unchanged. There is, however, some correlation be-

tween heart area and flow. The total circulating blood volume, as well as the volume in heart and lungs ( $V$ ) has a tendency to be increased and in both there is a fairly wide spread in values. The significant finding, however, is the striking linear relationship that some of the cases show between these two factors. In four out of six cases the change in total circulating blood volume exceeds the change in blood in the heart and lungs ( $V$ ) by 20 to 30 per cent. These findings seem to prove that in some cases at least the capillary cross section in the greater circulation is increased over that of the lesser circulation, and tend to uphold the contention of Dock and Tainter that the engorgement takes place in the liver or splanchnic vessels.

#### SUMMARY

1 In ten cases with apparently normal cardiovascular systems, the hemodynamics was studied before and twenty four hours after the oral administration of from one to one and two tenths grams of digitalis (digitoxin tablets).

2 By the use of the dye injection output method, it was possible to determine simultaneously the velocity of blood flow, the flow per minute, the total circulating blood volume, the volume of circulating blood in the lungs and heart ( $V$ ), the cell volume and the specific gravity of the plasma. It was possible also to obtain almost at the same time and certainly without any change in position of the patient, the venous and arterial blood pressures, the vital capacity and the cardiac silhouette area.

3 In all of the factors studied, it was found that digitalis may cause an increase, a decrease, or no change at all in the individual values. The median of any group may show a decided trend, nevertheless the spread about the initial normal was prominent. This was particularly true of the venous pressure and flow per minute. In the former the median value was 60 per cent of the initial normal, whereas one case was 150 per cent of the initial normal, in the latter, the median value was 88 per cent of the initial normal whereas one case was 121 per cent of the initial normal. When the venous pressure and the flow per minute are plotted against each other there exists a fairly close linear relationship in about 70 per cent of the cases.

4 There was evidence of some correlation between work and cardiac silhouette area, flow per minute and cardiac silhouette area, specific gravity of the plasma and the cell volume, and total circulating blood volume and the circulating blood volume in the lungs and heart. We believe the latter correlation is significant, in that it shows that digitalis in some cases exerts a peripheral action to cause an increase of circulating blood in the greater circulation, though not necessarily associated with changes one way or the other in the flow per minute, venous pressure or velocity of blood flow.

#### REFERENCES

- 1 Cohn, A. E., and Steele, J. M. Studies on the Effect of the Action of Digitalis on the Output of Blood From the Heart. 1 The Effect on the Output of the Dog's Heart in the Heart Lung Preparations, *J. Clin. Investigation* 11: 871, 1932.
- 2 Harrison, I. R., and Leonard, B. W. The Effect of Digitalis on the Cardiac Output of Dogs and Its Bearing on the Action of the Drug in Heart Disease, *J. Clin. Investigation* 3: 1, 1926.
- 3 Stewart, H. T., and Cohn, A. I. Studies on the Effect of the Action of Digitalis on the Output of Blood From the Heart, *J. Clin. Investigation* 11: 917, 1932.

4. Dock, W., and Tainter, M. L.: The Circulatory Changes After Full Therapeutic Doses of Digitalis With a Critical Discussion of Views on the Cardiac Output, *J. Clin. Investigation* 8: 467, 1930.  
Further Observations on the Circulatory Actions of Digitalis and Strophanthus With Special Reference to the Liver, and Comparison With Histamine and Epinephrine, *Ibid.* 8: 485, 1930.
5. Burwell, C. S., Neighbors, D. W., and Regan, E. M.: The Effect of Digitalis Upon the Output of the Heart in Normal Man, *J. Clin. Investigation* 5: 125, 1927.
6. Rytand, D. A.: The Effect of Digitalis on the Venous Pressure of Normal Individuals, *J. Clin. Investigation* 12: 847, 1933.
7. Quoted from Burwell and others.<sup>5</sup>
8. Quoted from Giordano, C., and Vigliani, E.: L'azione immediata della digitale sull'emodinamica, *Minerva med.* 2: 832, 1934.
9. Groseurth, G., and Bansi, H. W.: Das Verhalten des Kreislaufs bei Körperlicher Arbeit, *Klin. Wchnschr.* 11: 2022, 1932.
10. Wollheim, E.: Die Zirkulierende Blutmenge und ihre Bedeutung für Kompensation und Dekompensation des Kreislaufs, *Ztschr. f. klin. Med.* 116: 269, 1931.
11. Kinsman, J. Murray, Moore, J. W., and Hamilton, W. F.: Studies on the Circulation. I. Injection Method: Physical and Mathematical Considerations, *Am. J. Physiol.* 89: 322, 1929.
12. Moore, John Walker, Kinsman, J. M., Hamilton, W. F., and Spurling, R. G.: Studies on the Circulation. II. Cardiac Output Determinations: Comparison of the Injection Method With the Direct Fick Procedure, *Am. J. Physiol.* 89: 331, 1929.
13. Hamilton, W. F., Moore, John Walker, Kinsman, J. M., and Spurling, R. G.: Studies on the Circulation. IV. Further Analysis of the Injection Method, and of Changes in Hemodynamics Under Physiological and Pathological Conditions, *Am. J. Physiol.* 99: 534, 1932.
14. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. A. M. A.* 88: 91, 1927.

## THE RELATIONSHIP OF VITAMIN C TO THE HEMORRHAGIC DIATHESES\*

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THE isolation and preparation of vitamin C in pure form has stimulated interest in the antiscorbutic vitamin in many fields. The striking response of the hemorrhagic manifestations of scurvy to vitamin C therapy has led to the use of ascorbic acid in other types of hemorrhage. Favorable therapeutic effects of the intravenous administration of ascorbic acid have been reported in several types of capillary bleeding, including that due to hemophilia, thrombocytopenic purpura, Schonlein's purpura and metrorrhagia.<sup>1,2</sup> Our own experience in a series of individuals with capillary bleeding of various types has been difficult to evaluate. In many patients bleeding continued unabated after the intravenous administration of 100 mg of ascorbic acid. In others hemorrhage ceased within a few hours after the injection. In clinical observations of this type the *post hoc ergo propter hoc* argument is notoriously unreliable unless fortified by confirmatory evidence.

In the present study, clinical observations of hemorrhagic phenomena have been correlated with blood changes, capillary resistance, and the urinary excretion of vitamin C before and during the prolonged administration of large amounts of orange juice.

Harris and Ray<sup>4</sup> have shown that the administration of large test doses of vitamin C to normal individuals results in a marked increase in the urinary excretion curve if the diet has been adequate. In the "unsaturated," scorbutic, or semiscorbutic subject, however, the urinary excretion remains low until saturation has taken place. In this investigation, the excretion of a large proportion of ingested vitamin C has been assumed to indicate saturation of the tissues with antiscorbutic material.

Four patients with hemophilia and two patients with atypical chronic thrombocytopenic purpura were studied. After preliminary clinical and laboratory observations, each patient was given, in addition to the regular diet, a daily ration of orange juice equivalent to 100 or 200 mg of vitamin C. With one exception (C M), the usual diet had contained an average amount of antiscorbutic foods. Repeated observations of capillary resistance, twenty-four-hour excretion of vitamin C, and blood studies were made during the experimental period. Estimations of ascorbic acid in the urine were made by titration with a standardized solution of 2,6-dichlorophenolindophenol according to a method described by Harris and Ray.<sup>4</sup> Capillary resistance was determined with a modification of the apparatus described by Dalldorf.<sup>5</sup> The cup was applied to the inner surface of the upper arm for one minute, results

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were expressed as the amount of negative pressure necessary to produce macroscopic petechiae. The method of Lee and White was used in determining coagulation time. Bleeding time was determined by the method of Duke.

### CASE REPORTS

CASE 1.—J. K., a twenty-year-old youth, had had repeated frequent hemorrhages into joints, muscles, skin, and mucous membranes, and episodes of hematuria since birth. Both he and his brother, W. K., who also has hemophilia, have been studied and reported by Birch<sup>6</sup> with reference to the effect of ovarian preparations. Neither has had any glandular therapy since 1932.

The patient was admitted to the hospital on May 4, 1935, because of an acute hemarthrosis of the left knee and massive hematoma of the left thigh. The coagulation time was 187 minutes; retraction and character of the clot were normal. The bleeding time was two and one-half minutes. There was a moderate degree of hypochromic anemia; platelets were abundant. Capillary resistance was 10 cm. Hg. After obtaining a twenty-four-hour specimen of urine to determine the basal level of vitamin C excretion, daily administration of 200 c.c. of orange juice was begun. Although saturation with the vitamin

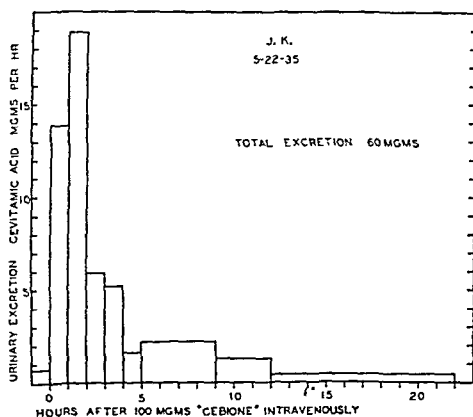


Fig. 1.—Hourly excretion of vitamin C after the intravenous administration of 100 mg. of cevitamic acid (Patient J. K.).

was accomplished (Table I), repeated hemorrhages into joints, muscle, and skin continued at intervals of two or three days. The capillary resistance remained unchanged. The intravenous administration of 100 mg. of cevitamic acid on May 22 resulted in a normal excretion curve (Fig. 1) and had no effect on the recurring joint and muscle hemorrhages. On May 28, after receiving 100 mg. of vitamin C daily for three weeks, a submucous hemorrhage into the larynx occurred, resulting in difficulty in breathing and swallowing, and in hoarseness. The coagulation time was 230 minutes; the capillary resistance had decreased to 6 cm. Hg. The intramuscular administration of 15 c.c. of normal human serum and the transfusion of 300 c.c. of citrated blood resulted in prompt cessation of the hemorrhagic manifestations for a period of about two weeks. Orange juice, which had been discontinued because of difficulty in swallowing, was resumed on June 15. During the next two months repeated hemorrhages occurred, requiring three transfusions. After each transfusion, bleeding temporarily ceased and the coagulation time diminished, on one occasion from 163 to 31 minutes, on another occasion from 180 to 15 minutes. It is apparent that in this patient saturation with orange juice and the administration of vitamin C intravenously had no effect on the bleeding, the coagulation time, or the capillary resistance.

CASE 2.—W. K., brother of J. K. At the age of six months a hemorrhage occurred in the cervical portion of the spinal cord, resulting in paralysis of all extremities for nine months. Neurologic residuals still persist in the form of ptosis of the left lid and scattered sensory

changes. The interval between hemorrhages of various parts of the body has never been more than a month. All extremities showed permanent deformity due to rigid joint hemorrhages. Two months before vitamin C studies were begun there had been a massive hemorrhage into the muscles of the left thigh. As this subsided over a small subcutaneous hemorrhages occurred.

On May 23, 1935, the clotting time was 10 minutes, the clot retracted in normal fashion. The bleeding time was three minutes. There was no anemia; platelets were abundant. Capillary resistance was 17 cm Hg. There had been no hemorrhagic manifestations for several days. Four hundred cubic centimeters of orange juice were added to the daily diet. Six days later, bleeding into the muscles of the right thigh began and continued for about ten days. The capillary resistance on three occasions during this period remained at 15 or 17 cm Hg. The excretion of large amounts of vitamin C in the urine indicated that saturation with the vitamin had taken place at the time the bleeding began (Table I). The coagulation time on June 15, after almost three weeks of high vitamin C intake, was 130 minutes. Although orange juice was continued during the next two months, intermittent bleeding occurred from time to time. During this period two transfusions were required to control major hemorrhagic episodes.

**CASE 3—W. P.**, a thirty-seven year old man had had repeated attacks of hematuria and hemorrhage into joints and muscles since early childhood. Both he and his brother were

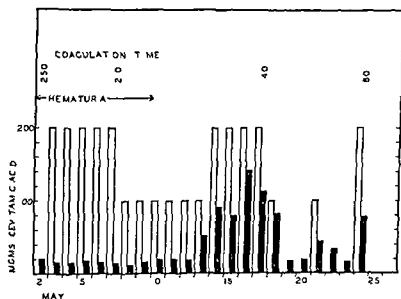


Fig 2—The daily intake and excretion of vitamin C in a patient with hemophilia (W. P.). The open columns represent cevitamic acid taken in the form of orange juice. The solid columns represent the twenty-four hour excretion of cevitamic acid in the urine. The coagulation time is given in minutes.

known hemophiliacs. A maternal uncle had died of hemorrhage following an injury. April 30, 1935, the patient was readmitted to the hospital because of hematuria of ten days' duration. The urine contained gross blood and some albumin but showed no casts. There was no anemia. Blood coagulation time was 250 minutes, clot retraction was normal. Bleeding time was two and one half minutes. Capillary resistance was 7 cm Hg and remained between 7 and 10 cm Hg during the period of observation. The intake and urinary excretion of vitamin C are shown in Fig 2. No other therapy was given. The urine began to clear within a few days, there was no evidence of microscopic or macroscopic hematuria after May 9.

It is apparent from the chart that the hematuria cleared and the coagulation time decreased somewhat during the period of vitamin C administration. However, it cannot be stated with certainty that vitamin C was responsible for the improvement observed, inasmuch as previous episodes of hematuria had cleared within a comparable or shorter period of time without antiscorbutic therapy. It should be noted that the urinary excretion of cevitamic acid did not begin to increase until several days after the urine had completely cleared. Orange juice was continued after the patient was discharged from the hospital, on June 4, the coagulation time had increased from 80 minutes to 130 minutes. A few days later there was bleeding into the subcutaneous tissues of the scrotum.

The significance of the vitamin C excretion curve is not clear. Saturation apparently did not occur until after large amounts of orange juice had been given for ten days. In the majority of normal individuals, even after a diet partially deficient in vitamin C, increased excretion of cevitamic acid begins within two or three days after the addition of large amounts of orange juice to the diet. In this patient, after saturation had occurred, the response to alternate deprivation and addition of vitamin C was similar to that which has been observed in normal subjects. It is possible that factors as yet uninvestigated may have been responsible for the apparent delay in saturation. A similar, but more prolonged, delay in the excretion of cevitamic acid has been observed in a patient with hematuria due to acute hemorrhagic nephritis.

TABLE I

PATIENT	TWENTY-FOUR-HOUR EXCRETION OF CEVITAMIC ACID IN MILLIGRAMS			
	CONTROL	DURING ADMINISTRATION OF ORANGE JUICE		
	REGULAR DIET	SEVENTH DAY	FOURTEENTH DAY	TWENTY-FIRST DAY
J. K.	5.2	95.5	60.0	-
W. K.	11.7	121.0	136.0	128.5
W. P.	14.3	16.9	141.0	73.5
C. M.	31.2	57.4	-	72.5
F. M.	12.4	34.7	30.6	30.6
R. T.	19.0	49.5	105.0	-

CASE 4.—C. M., a twenty-eight-year-old man, was admitted to the hospital because of persistent bleeding following tooth extraction ten days before. A previous tooth extraction had been followed by persistent hemorrhage of ten days' duration. There had been no other significant hemorrhagic phenomena. His usual diet contained very little fresh fruit or leafy vegetables. The patient's brother, one maternal uncle, and three male maternal cousins were said to be "bleeders." The physical examination was essentially negative except for mild oozing of blood from the tooth socket. A moderate degree of hypochromic anemia was present. Platelets were abundant. Coagulation time was twenty-five minutes; clot retraction was normal. Bleeding time was six minutes. A diagnosis of mild hemophilia was made on the basis of the blood findings and the family history.

A compound pressure splint was applied to the bleeding area and 100 mg. of cevitamic acid were administered intravenously. Bleeding ceased within four hours.

Four days later 200 c.c. of orange juice were added to the daily diet. One week later, the bleeding time was six minutes, coagulation time twenty minutes. After three weeks of high vitamin C intake, the coagulation time was forty minutes. The anemia improved without other therapy, and the patient gained weight. He volunteered the information that since taking orange juice he had felt better than for many years. There was no change in the capillary resistance during the period of observation. The improvement in general health, the gain in weight, and the improvement in the anemia after adding orange juice to the diet, considered with the previous dietary history, suggest that this patient may have had mild vitamin C deficiency. Saturation with vitamin C, however, had no demonstrable effect on the coagulation time or the capillary resistance.

CASE 5.—R. T., a twenty-one-year-old man, had been known to have chronic thrombocytopenic purpura for at least three years. In addition to intermittent bleeding, there had been episodes of characteristic agranulocytic angina in 1932 and in 1933. In May, 1935, he had been free of hemorrhagic manifestations for about one year. During this period, however, there had been persistent thrombocytopenia, without other demonstrable morphologic blood abnormalities. On May 28, hematologic observations were as follows: hemoglobin, 16.2 gm. per 100 c.c.; red blood cells, 5,600,000 per c. mm.; white blood cells, 4,360 per c. mm., of which 58 per cent were neutrophilic granulocytes. Platelets were markedly diminished. Bleeding time was five and one-half minutes. Coagulation time was fifteen minutes; after twenty-four hours there was no retraction of the clot, which was very friable. Capillary resistance was 12 cm. Hg. Saturation with vitamin C was accomplished (Table I) by the addition of 400 c.c. of orange juice to the daily diet. After two weeks, the coagulation time was

nineteen minutes, the clot did not retract. Bleeding time was six minutes. No significant change had taken place in the hemoglobin, red blood cells, white blood cells or platelets. During the period of observation, the capillary resistance remained between 12 and 16 cm Hg.

CASE 6—F M, a sixty-one year old carpenter had been known to have chronic thrombocytopenic purpura for at least five years. His clinical course had been characterized by persistent granulocytopenia of varying degree and by episodes of bleeding and anemia. For at least a year prior to the vitamin C studies there had been frequent episodes of bleeding from the nose and gums, interspersed with showers of petechiae and traumatic ecchymoses, so that at no time had he been free of hemorrhagic manifestations of one kind or another. On May 28, 1935, there were scattered, fresh petechiae in several areas and a recent ecchymosis on the right arm. Blood hemoglobin was 12.5 gm per 100 cc, red blood cells numbered 4,430,000 per c mm, white blood cells, 920 per c mm. Differential count was as follows: eosinophiles, 1 per cent, stab neutrophils, 1 per cent, segment neutrophils 26 per cent, lymphocytes, 69 per cent, monocytes, 3 per cent. The platelets were markedly diminished. Bleeding time was eleven and one half minutes. Coagulation time was nineteen minutes. There was no retraction of the clot in twenty four hours, the clot was flabby and friable. Capillary resistance was 9 cm Hg. The daily administration of 400 cc of orange juice resulted in the excretion of almost three times as much vitamin C as during the control period, indicating at least partial saturation with the vitamin. Frequent showers of petechiae and small ecchymoses continued to occur. If anything, hemorrhagic manifestations were more marked during the period of orange juice administration. After two weeks of forced vitamin C feeding, the blood findings were as follows: hemoglobin, 12.6 gm per 100 cc, red blood cells, 5,010,000 per c mm, white blood cells, 1,160 per c mm. Platelets remained markedly diminished. Bleeding time was twelve minutes. Coagulation time was twenty three minutes, without change in the character of the clot. During the period of observation the capillary resistance remained at 9 or 10 cm Hg. High vitamin C diet was continued for three months without change in the frequency or severity of the skin and mucous membrane bleeding.

## DISCUSSION

Table I presents evidence that saturation with vitamin C was accomplished in each of the six individuals studied, with the possible exception of F M. The apparent delay in saturation observed in W P indicates the need for further investigation of the factors which determine vitamin C excretion. Patients with hemophilia and with thrombocytopenic purpura apparently do not differ from normal individuals in their ability to excrete large amounts of ingested vitamin C. Confirmatory evidence of this is found in the report of Schroeder<sup>7</sup> who observed a normal response to the administration of 300 mg of ascorbic acid in one patient with hemophilia. Finkle,<sup>8</sup> on the other hand, reports low values for vitamin C excretion in five cases of thrombocytopenic purpura and in four cases of meliorrhagia. It should be noted, however, that the previous dietary history of these patients is not mentioned and that results are expressed in terms of urinary concentration of vitamin C, rather than as the total amount excreted.

It is apparent from the case reports that saturation with vitamin C had no demonstrable effect on the hemorrhagic manifestations. In three of the patients with hemophilia and in one patient with thrombocytopenic purpura, hemorrhages recurred or continued during the period when large amounts of orange juice were being given. The other two patients were in symptomatic remission at the time the observations were made. No significant changes in the number of platelets, bleeding time, clotting time, character of the clot, or

in capillary resistance were observed. These results suggest that there is no characteristic abnormality of vitamin C metabolism and no constant therapeutic effect of antiscorbutic material in hemophilia or thrombocytopenic purpura.

It has been shown that the primary disturbance responsible for the hemorrhagic manifestations of scurvy is a separation of the cement substances binding the vascular endothelial cells.<sup>9</sup> Wolbach and others<sup>10, 11</sup> have demonstrated that the immediate effect of the administration of orange juice or ascorbic acid in experimental scurvy is the prompt deposition of intercellular material. Preservation of the integrity of the intercellular cement substance is apparently a specific function of the antiscorbutic vitamin. It is noteworthy that disturbances of the intercellular cement substance have not been described in hemorrhagic lesions other than those due to scurvy. Theoretically, therefore, vitamin C might be expected to have no effect on the capillary bleeding of the other hemorrhagic diatheses unless an associated vitamin deficiency of some degree were present.

Instances of capillary hemorrhage due to mild or "subclinical" scurvy may be more frequent than has hitherto been suspected. We have recently observed three individuals with gingival bleeding thought to be due to relative vitamin C deficiency. Consumption of vitamin C containing foods had been curtailed because of special diets or individual preferences. In each instance there was a history of bleeding from the gums, occurring both spontaneously and after brushing. The gums appeared dusky, mottled, soft, and edematous. There were no symptoms or signs of hemorrhage from other sites. Bleeding time, coagulation time, blood counts, and platelets were normal. Large amounts of vitamin C were supplied in the form of 400 c.c. of orange juice daily. Although there was no significant change in the capillary resistance, the gingival bleeding diminished almost immediately and ceased entirely within a few days. Cessation of the bleeding was accompanied by an increase in the urinary excretion of cevitamic acid. In two of these individuals the bleeding returned when orange juice was omitted from the diet, disappeared when large amounts of vitamin C were again given. In another instance the subcutaneous hemorrhages, which had regularly followed desensitizing doses of pollen extract given to a young woman with hay fever, permanently disappeared when adequate amounts of orange juice were added to the diet.

There are undoubtedly other types of capillary bleeding which may be associated with inadequate vitamin C intake. Hemorrhages in infantilism and marasmus have been observed as a consequence of vitamin deprivation.<sup>9</sup> The "symptomatic" or "simple" purpuras, in which morphologic blood changes are not demonstrable, should be studied from this point of view. Many patients with gastrointestinal and other diseases are given diets which contain inadequate amounts of fresh fruits and leafy vegetables. Such individuals might easily develop a relative vitamin C deficiency unless care is taken to provide orange juice or other suitable antiscorbutic material. The possible relationship of the bleeding of peptic ulcer to vitamin deficiency has not been investigated. Harris and Ray<sup>4</sup> have suggested a test for subclinical scurvy which

depends on the urinary excretion of cevitamic acid after a massive test dose of vitamin C. The results of the application of this test to various types of capillary bleeding will be of interest.

# SUMMARY

1 The effect of large doses of orange juice has been studied in four patients with hemophilia and in two patients with chronic thrombocytopenic purpura.

2 Saturation with vitamin C had no effect on the hemorrhagic manifestations, the blood picture, or the capillary resistance.

3 It is suggested that favorable therapeutic effects may be expected from the administration of vitamin C only in those instances of capillary bleeding which are associated with some degree of vitamin C deficiency.

NOTE: Since the preparation of this communication, Wright and Lilienfeld<sup>12</sup> have reported observations on the effect of the administration of cevitamic acid to three patients with purpura and two patients with hemophilia. They expressed the opinion that its value in the treatment of thrombocytopenic purpura or familial hemophilia is doubtful. Miller and Rhoads<sup>13</sup> observed a persistent rise in the number of thrombocytes and complete relief of symptoms following the administration of cevitamic acid to four patients with idiopathic thrombocytopenic purpura. In two instances, clinical improvement and increase in the number of thrombocytes were associated with an increased urinary output of ascorbic acid.

The authors wish to express their appreciation to Dr. Samuel W. Clausen for his interest in this study.

# REFERENCES

- 1 Boger, A., and Schroeder, H. Vitamin C und Plasmerückbildung, *Klin. Wchnschr.* 13: 842, 1934.
- 2 Boger, A., and Schroeder, H. Ueber die Stillung schwerster Blutungen bei allen Formen der hämorrhagischen Diathese und der Hemophilie durch parenterale Zufuhr von C-Vitamin, *München med. Wchnschr.* 81: 1335, 1934.
- 3 Junghans, E. Die Behandlung von gynäkologischen Blutungen mit Vitamin C, *Klin. Wchnschr.* 14: 899, 1935.
- 4 Harris, L. J., and Ray, S. N. Diagnosis of Vitamin C Subnutrition by Urine Analysis, *Lancet* 1: 74, 1935.
- 5 Dilldorf, G. A Sensitive Test for Subclinical Scurvy in Man, *Am. J. Dis. Child.* 46: 794, 1933.
- 6 Birch, C. L. Hemophilia, *J. A. M. A.* 99: 1566, 1932.
- 7 Schroeder, H. Die Ausscheidung der Ascorbinsäure im gesunden und kranken Organismus, *Klin. Wchnschr.* 14: 484, 1935.
- 8 Fink, J. Urinary excretion of Vitamin C in Some Vascular Diseases, *Proc. Soc. Exptl. Biol. Med.* 1163, 1935.
- 9 Kugel, H. Control of Chronic Hemorrhagic States in Childhood, *Am. J. Dis. Child.* 46: 794, 1933.
- 10 Wolbach, S. B., and Howe, P. R. Intercellular Substances in Experimental Scurvy, *Arch. Path.* 1: 1, 1926.
- 11 Menkin, V., Wolbach, S. B., and Menkin, M. F. Formation of Intercellular Substance by the Administration of Ascorbic Acid (Vitamin C) in Experimental Scorbute, *Am. J. Path.* 10: 569, 1934.
- 12 Wright, I. S., and Lilienfeld, A. Pharmacologic and Therapeutic Properties of Crystalline Vitamin C (Cevitamic Acid) With Special Reference to the Effects on the Capillary Fragility, *Arch. Int. Med.* 57: 241, 1936.
- 13 Miller, D. K., and Rhoads, C. P. Ascorbic Acid in the Treatment of Thrombocytopenic Purpura, *J. Clin. Investigation* 15: 462, 1936.

# A STUDY OF DECAMETHYLENEDIGUANIDINE BITARTRATE (ANTICOMAN)\*

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IN 1931 and 1932, there appeared in the German literature<sup>1</sup> descriptions of a proprietary substance called "anticoman" recommended to be administered orally in the treatment of diabetes mellitus, to reduce hyperglycemia. Prior to this time, attempts<sup>2</sup> had been made to produce a substance which, if administered orally, would have the same effect as insulin. Many of these products have been shown to be rather toxic or ineffective. A well-known example of these attempts is "synthalin," which is a guanidine preparation.

In 1933, the chemical nature of "anticoman" was revealed to be decamethylenediguanidine bitartrate. It is soluble in water and gastric juice, and may be prepared by first making a basic compound by melting a polymethylenebisguanidine with the rhodonate of guanidine at 130° to 160° C. and treating with alkali hydrate or carbonate. These basic compounds are then caused to react with tartaric acid.<sup>3</sup>

## TOXICITY FOR MICE

*Method.*—We first used a water solution of decamethylenebisguanidine bitartrate, the concentration of which was 5 mg. of the salt in 1 c.c. of the solution. In the experiments, we used white mice weighing between 15 and 20 gm. All injections were made subcutaneously. The mice were weighed daily, and the injections adjusted accordingly. Results are found in Table I.

TABLE I

MOUSE	DOSE	DOSE IN MILLIGRAMS PER 100 GM. OF MOUSE	RESULTS
1	1 c.c.	34 mg.	Dead in 2 hours
2	0.75	23 mg.	Dead in 30 minutes
3	0.50	15 mg.	Dead in 1 hour 15 minutes
4	0.25	7 mg.	Dead in 2 hours

Using a solution which contained 1.25 mg. of the salt in 1 c.c., we obtained the results as shown in Table II.

*Discussion.*—When large doses are given, as in Mice 1 to 4 inclusive, about one hour previous to death, a convulsive type of reaction could be elicited by the slightest stimulus. This may be similar to the symptom complex observed in cats, produced by dimethyleneguanidine, and almost identical with spas-

\*From the Laboratory of Pharmacology, University of Illinois College of Medicine.  
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mophulia in children. This might be explained on the basis that guanidine produces symptoms similar to parathyroid tetany.<sup>4</sup> From the results obtained with mice, it is to be noted that 2.25 mg. of decamethylenediguanidine bitartrate per 100 gm. of mouse can be injected and the animal will remain alive for at least twenty-four hours. However, a dose of about 1.0 mg. of salt per 100 gm. of mouse is the largest dose that can safely be injected daily for any appreciable period of time.

TABLE II

MOUSE	DOSE	AVERAGE DOSE IN MG. PER 100 GM. OF MOUSE		RESULTS
8	1.0 c.c.	0.5 mg.		Dead in 3 hours
7	0.75	5.7		Dead in 20 hours
6	0.5	3.1		Dead in 20 hours
12	0.3	2.24		Dead in 48 hours
5	0.25	1.8		Living
11	0.3	2.5	Injected daily for two days	Died
10	0.3	2.2	Injected daily for three days	Died
9	0.3	2.0	Injected daily for three days	Died
17	0.3	2.2	Injected daily for two days	Died
16	0.3	2.0	Injected daily for three days	Died
21	0.3	2.0	Injected daily for three days	Died
13	0.3	1.8	Injected daily for five days	Died
14	0.15	1.3	Injected daily for four days	Living
15	0.3	2.2	Injected on alternate days	Living after 3 injections
18	0.3	1.9	Injected on alternate days	Died after 3 injections
19	0.15	0.95	Injected daily for 17 days	Died
20	0.15	1.04	Injected daily for 17 days	Died

*Conclusions from these experiments on mice:* The largest dose of decamethylenediguanidine bitartrate that can be used for subcutaneous injections is 2.25 mg. per 100 gm. of mouse for a single injection; 1.0 mg. per 100 gm. of mouse for repeated daily injection.

#### TOXIC AND GLYCEMIC EFFECTS ON NORMAL RABBITS

*Methods.*—The rabbits used in these experiments were starved for a period of twenty-four hours previous to the administration of the drug, and no food was given during the experiment. The drug was administered through a stomach tube. One cubic centimeter of blood was drawn from the marginal ear vein for sugar determination, a modification of the Folin-Wu method being used.<sup>5</sup> Results are found in Table III.

The effect of the salt on the blood sugar of rabbits when injected subcutaneously was then observed. A 4 per cent solution was used. Results are found in Table IV.

*Observations.*—Rabbits 17 and 29 died in convulsions when their blood sugar dropped to 36 and 22 mg., respectively. Microscopic sections were made of the kidneys and livers of Rabbits 15, 17, and 29. Tissues were taken immediately after death.

TABLE III

RABBIT	WT. IN KG.	DOSE PER KG.	NORMAL	BLOOD SUGAR IN MG. PER 100 C.C.									
				1 HR.	2	3	4	5	7	24	28	54	
1	2.2	Control	105	81	91	80					140		
2	2.0	20 mg.	118	85	94	72					85		
3	2.4	20	111	103	112	96					69		
4	2.3	Control	119					99	117				
5	1.9	20	113					93	103				
6	2.5	20	133					129	129				
7	2.6	Control	129	110	102		120	116			94	91	
8	3.0	24	116	120	118		105	109			98	89	
9	2.3	24	120	112	116		115	107			95	96	
10	2.6	24	127	108	120		110	113			94	84	
11	2.5	30	110	98	113		104	113			86	82	
12	2.3	30	119	102	111		114	93			75	76	
13	1.6	40	104	78			83					83	
14	2.8	40	98						86		99		
15	1.4	50	100	58			57		Died				
16	2.0	50	101						80.3			Died	

TABLE IV

RABBIT	WT. IN KG.	DOSE PER KG.	NORMAL	BLOOD SUGAR IN MG. PER 100 C.C.													
				1 HR.	2	6	8	15	17	21	22	23	24	26	28	32	42
17	3.0	6 mg.	123	147							50		36				
18	3.1	9	109	136													
19	2.8	Control	89	94							109						
20	2.4	3	104		121	130	145							108	96	88	
21	3.1	5	106		119	102	132							112	121	111	
22	2.8	Control	105		95	98	121							104	97	100	
23	2.1	Control	105					90	88	97		104					94
24	2.3	3	103					118	129	125		125					125
25	2.8	5	98					114	94	108		101					116
26	2.3	5	110	146	106	119							115	128		127	100
27	3.2	6	108	121	77	113							106	104		119	131
28	2.4	7	104	141	101	92							73	77		74	121
29	2.0	9	103	51	22												

*Microscopic examination* revealed a marked necrosis and desquamation of the tubular epithelium of the kidney; a condition similar to that observed in mercuric poisoning. This was especially noted in the tissue from Rabbit 15. Sections from the livers did not show any pathology. Tissues were stained by hematoxylin and eosin.

## SUMMARY

The results as shown in Table III indicate that there is little effect on the blood sugar when small doses of the salt are administered orally. However, with a dose of 50 mg. of anticoman per kilogram of rabbit, the blood sugar dropped markedly, and the rabbits died. The next smaller dose of 40 mg had only a very slight hypoglycemic effect. This seems to indicate that the glycemic effect

is obtained only when the toxic dose is reached. In Table IV, the results show that doses up to and including 5 mg of decamethylenediguanidine bitartrate per kilogram of rabbit subcutaneously have practically no hypoglycemic effect on normal rabbits when observed over a period of forty-eight hours. When the dose is raised to 6 mg of salt per kilogram of rabbit the hypoglycemic effect may be so great as to produce convulsions and death, or there may be only a slight hypoglycemia, apparent after six hours, but this disappears by the eighth hour. Above 6 mg, marked hypoglycemia is produced and the animal dies in convulsions.

#### CONCLUSIONS FROM EXPERIMENTS ON RABBITS

1 On oral administration, 50 mg of decamethylenediguanidine bitartrate per kilogram of normal are lethal. Forty milligrams produce a slight hypoglycemia, smaller doses have practically no effect.

2 On subcutaneous injections, doses over 6 mg per kilogram are effective in producing hypoglycemia but are fatal. Six milligrams in some cases may or may not produce hypoglycemia, but below this dose, the salt is ineffective.

3 Histologic examination shows that the effect of the drug is to produce a tubular nephritis, similar to that obtained in mercuric poisoning.

4 From these results, we cannot expect the drug to be of value in clinical cases.

#### REFERENCES

- 1 Frohlich, F. *Med Klin* 28 1556, 1932  
Bernheim, E, and Kaszsis, T. *Fortschr d Med* 50 171, 1932  
Grote, L. R. *Deutsche med Wchnschr* 57 2099, 1931  
Kahnt, K. *Med Welt* 5 886, 1932
- 2 Frank, E, and Wagner, A. *Wurzb Abhandl u d Gesamtgeb Med* 27 255, 1932  
Thomson, A. P., Gittins, and Thomas, G. *Brit M J* 1 322, 1932  
Eismayer, G. *Klin Wchnschr* 11 860, 1932
- 3 Chem Abstracts 1933 1006, *Anticom Ges mbH* Fr 735,087
- 4 Frank, Stern, and Nothmann. *Exper Med* 24 271, 1921
- 5 Benedict. *J Biol Chem* 76 457, 1928

# LABORATORY METHODS

## THE PRACTICAL VALUE OF EMPLOYING MORE THAN ONE LABORATORY PROCEDURE IN THE SERODIAGNOSIS OF SYPHILIS\*

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FOR some time we have been employing Kolmer's 2-tube complement fixation method as a routine procedure, together with Kahn's standard test upon request, in the serodiagnosis of syphilis. To date we have accumulated comparative reactions on more than 50,000 specimens.

It is our purpose to set forth in this paper these comparisons with the view of presenting the practical value of employing more than one kind of procedure. No attempt has been made to obtain the diagnoses of the clinicians with the exception of the first 10,000 specimens under consideration.

It is well, we think, to describe briefly the procedure employed. Although appreciating the superiority of Kolmer's more recent 3-tube method, the volume of our work, together with limitation of personnel and equipment, has necessitated the continuation of his former method. Serum inactivated for thirty minutes is employed in doses of 0.1 c.c. Natural antishoop hemolysin is not removed. Titrations of both hemolysin and complement (1-30) are made daily. Ten units of antigen are used. Primary incubation is conducted at 6° to 8° C. for fourteen to eighteen hours followed by water-bath for ten to fifteen minutes. Secondary incubation is in water-bath for one hour. Reactions are read immediately.

The Kahn 3-tube standard test is performed on the same day the complement fixation test is set up. Standardized antigen is obtained from the Michigan Department of Health.

TABLE I  
CLASSIFICATION OF WASSERMANN AND KAHN REACTIONS ON 50,000 SPECIMENS

WASSERMANN							
KAHN		4+	3+	2+	1+	±	Neg.
	4+	6157	203	114	165	77	277
	3+	861	88	120	101	51	305
	2+	208	51	82	122	38	375
	1+	96	18	42	86	43	306
	±	65	7	10	24	38	203
	Neg.	215	39	66	105	92	40,532

\*From the Department of Serology, Georgia State Department of Health.  
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In cases of disagreement both tests were repeated in order to rule out technical error. Table I gives the results of these comparative examinations in tabulated form. It will be noted that 277 specimens showed four plus Kahn and negative Wassermann, whereas 215 specimens gave four plus Wassermann and negative Kahn reactions.

It must be borne in mind that the higher the percentage of positive reactions encountered in routine work, the higher the discrepancy in reactions is likely to be. We would not expect to find as many disagreements of results in nonsyphilitic as in syphilitic patients. The average percentage of positive reactions in routine examinations over the period of time covered in this study ranged from 18 to 20 per cent. Employing the same procedures, laboratories would probably find fewer or more discrepancies dependent upon the average percentage of positive reactions.

We find that almost 1 per cent of the 50,000 specimens showed four-plus by one and negative by the other test, with the Kahn showing slightly more positives. These represent about 8 per cent of the four plus reactions in which both tests were in agreement.

TABLE II  
SUMMARY OF DISAGREEMENTS BETWEEN THE WASSERMANN AND KAHN TESTS

Positive Kahn—negative Wassermann	957
Positive Wassermann—negative Kahn	320
Positive Kahn—doubtful Wassermann	554
Positive Wassermann—doubtful Kahn	238
Doubtful Kahn—negative Wassermann	509
Doubtful Wassermann—negative Kahn	197

The disagreements are summarized in Table II by the usually accepted scheme of representing four, three, and two plus reactions as positive, and one-plus and plus minus as doubtful. The Kahn test gives the greater number of positive reactions.

Mention was made above that we sought a study of the clinical data in the disagreements encountered in the first 10,000 specimens. Questionnaire cards relating to the history and symptoms were attached to the reports. The physicians cooperated well indeed, returning 93.4 per cent of the cards. Clinical diagnoses usually pointed to the positive results in these cases of disagreement between the two tests.

TABLE III  
ANTICOMPLEMENTARY REACTIONS

KAHN	4+	3+	2+	1+	±	~	TOTAL
	302	69	38	8	10	122	549

#### ANTICOMPLEMENTARY REACTIONS

The Kahn test was made on all anticomplementary Wassermann reactions encountered in routine work during this study. Table III gives the results of these reactions. It will be noted that the vast majority of the anticomplementary reactions gave positive Kahn results. This causes one to wonder as to the rela-

tionship of such to specific reactions. In our experience the majority of anti-complementary reactions obtained with two units of complement yield apparently positive reactions when repeated with three units of complement and are positive by the Kahn test.

TABLE IV

SHOWING CONSISTENCY OF RESULTS OBTAINED WITH WASSERMANN AND KAHN TESTS  
IN DIFFERENT GROUPS OF SPECIMENS

	FIRST 10,000	NEXT 20,000	NEXT 20,000	TOTAL 50,000
1. <i>Identical Agreement</i> No variation in terms of plus	91.1	91.4	91.6	91.4
2. <i>Complete Agreement</i> 4+, 3+, and 2+ = positive. 1+ and ± = doubtful. Positive, doubtful or negative by both tests	94.2	94.4	94.9	94.6
3. <i>Relative Agreement</i> Positive or negative by one test and doubtful by the other	3.4	2.8	2.8	2.9
4. <i>Disagreements</i> Positive by one test and negative by the other	2.4	2.8	2.3	2.5

Table IV is presented to demonstrate the consistency of the agreement between the two tests employed. In the first column are given the percentages for the first 10,000 specimens. The second column represents the following 20,000 and the third column the last 20,000 specimens. The fourth column gives the average for the entire group.

It will be noted that there is less than 1 per cent variation in each of the groups presented. We feel that this consistency is significant of the result of controlling one test by means of another. There have been instances when we found the results by the two tests diverging more than usually. Investigation revealed the trouble somewhere along the line, which, when remedied, resulted in a return to the usually expected agreement. These difficulties have been experienced in the performance of both the complement fixation and Kahn tests.

We believe that herein lies the practical value to the laboratory in employing more than one procedure. There may be little to choose as to efficiency between a good complement fixation and a good flocculation method, provided they are carefully controlled. The question is, what is the best method to control them? The controls set up with each test are highly desirable, but they may not reveal errors of technicians or gradual changes in reagents. For instance, if an antigen should lose antigenic properties or become anticomplementary, it is reasonable to suppose these changes develop gradually and may exert influence over the results of the test before they are detected by the controls. Then again the specificity of a new lot of antigen may vary from that of the lot in use, even though the titer with a particular pooled serum may be as good or even superior.

The employment of two tests routinely, as recommended by the Health Organization of the League of Nations, and others, is to be desired. However, some laboratories find it impractical to execute these recommendations because of economic reasons. The preference of tests to use routinely and for check pur-

poses will usually rest with the serologist as determined by his experience. It occurs to us that this purpose can best be served by choosing a representative test from both the fields of complement fixation and flocculation.

Our conclusion is that aside from the added information the laboratory may be able to give the physician the employment of an additional procedure, at least to such an extent that it will serve as a check and control on the other, is conducive to better work in the routine serodiagnosis of syphilis. This conclusion, of course, is based upon the assumption that conditions will permit the employment of a supplementary test without in any way detracting from the usual care and accuracy demanded in serologic work.

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## A PRACTICAL METHOD OF MEASURING AND RECORDING SKIN TEST REACTIONS\*

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THE interpretation of skin tests is generally based on size in centimeters and degree of inflammatory reaction. The methods of determining the size of skin reactions have been most diverse, varying from the employment of rulers and calipers to mere guesswork. The method of recording has been usually word description or the employment of positive and negative signs. It has been our purpose to devise a simplified uniform method of measurement and recording with the view of increasing the rapidity, accuracy, and efficiency of such interpretation. While we have employed the following technique only in regard to Schick and Dick reactions, it could easily be adapted to any form of skin testing.

*Procedure*—A thin glass 4 cm. square is used upon which there are three circles. The small inner circle is 1 cm. in diameter, the second circle 2 cm. in diameter, and the outer and largest circle is 3 cm. in diameter. The glass is placed over the site of reaction so that the small circle is in the middle of the reacting area. By looking through the glass at the skin, the extent of the reaction is immediately given in terms of centimeters. The appearance of the glass is seen in Fig. 1.

In recording the reaction, a rubber stamp (see Fig. 1) is used which contains three circles of the same size as described above. Each time a reading is made, by use of the stamp, a diagram is imprinted on the card of the patient and the date of reading recorded below the diagram. The reaction is recorded schematically on the diagram by the employment of an arbitrary standard (see Fig. 2).

Using the standard glass, therefore, and the standard of color interpretation, an accurate schematic representation may be immediately transferred to the patient's record which can readily be visualized at any time, and can more easily be visualized than a word description of the same reaction. One who had

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not seen the patient could easily determine the type of reaction by a very rapid scanning of the patient's record inasmuch as there would immediately meet his eye a daily schematic representation of the reaction which could be correlated at a glance.

For illustration, and to exemplify the method of recording, duplications of actual Schick reactions are given in Fig. 3 and Dick reactions in Fig. 4. An associated word interpretation will be given in a few cases, which, of course, is not necessary when one is familiar with the method.

The record of Case 1 in Fig. 3 would be interpreted as follows: At the end of twenty-four hours there was a dark pink area 0.5 cm. in diameter surrounded by a light pink area 2 cm. in diameter. At the end of forty-eight hours, the dark pink area had increased to 1 cm. and the light pink area to 3 cm.

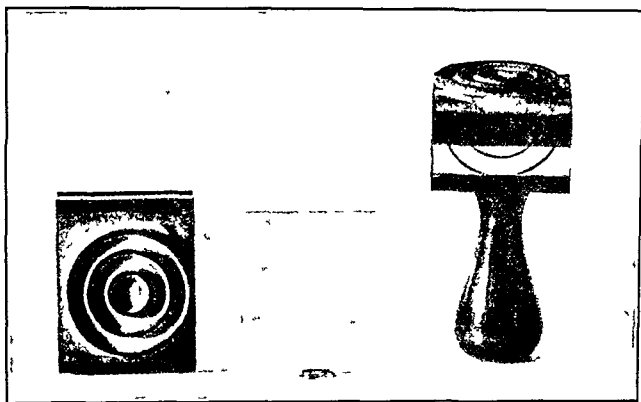


Fig. 1.—The measuring glass and recording stamp



Fig. 2.—Schematic standard employed in recording color reactions

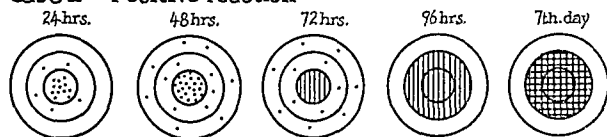
At the end of seventy-two hours the 1 cm. dark pink area became a deep red and the light pink area remained approximately the same. At the end of ninety-six hours, the light pink area had faded but the deep red area had increased to 2 cm. At the end of the seventh day there was a 2 cm. brownish red pigmented area.

The record of Case 4 in Fig. 3 would be interpreted as follows: At the end of twenty-four hours there was a dark pink area 0.5 cm. in diameter surrounded by a light pink area 3 cm. in diameter. At the end of forty-eight hours the dark pink area had become red and increased to 1 cm. in diameter. The light pink area had become dark pink and increased to 3.5 cm. At the end of seventy-two hours there remained only a light pink area 3.5 cm. in diameter. At the end of ninety-six hours there was complete fading.

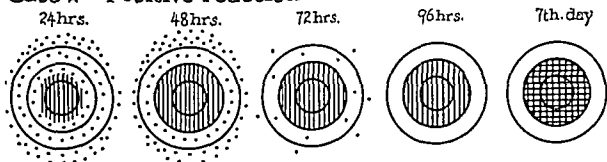
In Fig. 4 the records would be interpreted as follows: Case 1, a light pink area 2 cm. in diameter; Case 2, a dark pink area 1.5 cm. in diameter; Case 3, a dark pink area 3 cm. in diameter; Case 4, a dark pink area 2 cm. in diameter.

## RECORDS OF SCHICK REACTIONS.

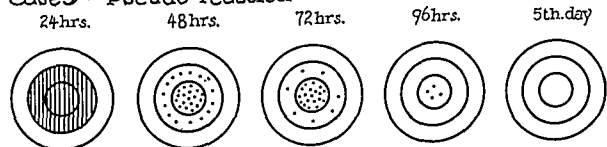
## Case 1 - Positive reaction



## Case 2 - Positive reaction



## Case 3 - Pseudo reaction



## Case 4 - Pseudo reaction

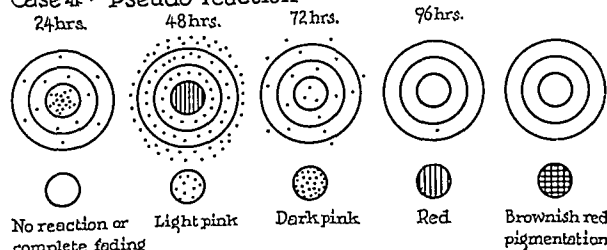


Fig. 3.

## DISCUSSION

The above-described method of measuring and recording skin tests (Schick and Dick reactions) has been used by us for three years on medical students with success. In 1934 this method was employed by the Division of Communicable Diseases of the Illinois Department of Public Health in Schick testing 140 children with excellent results. In 1933 in order to see whether the apparatus employed by two individuals would produce uniform results in the same

## RECORDS OF DICK POSITIVE REACTIONS

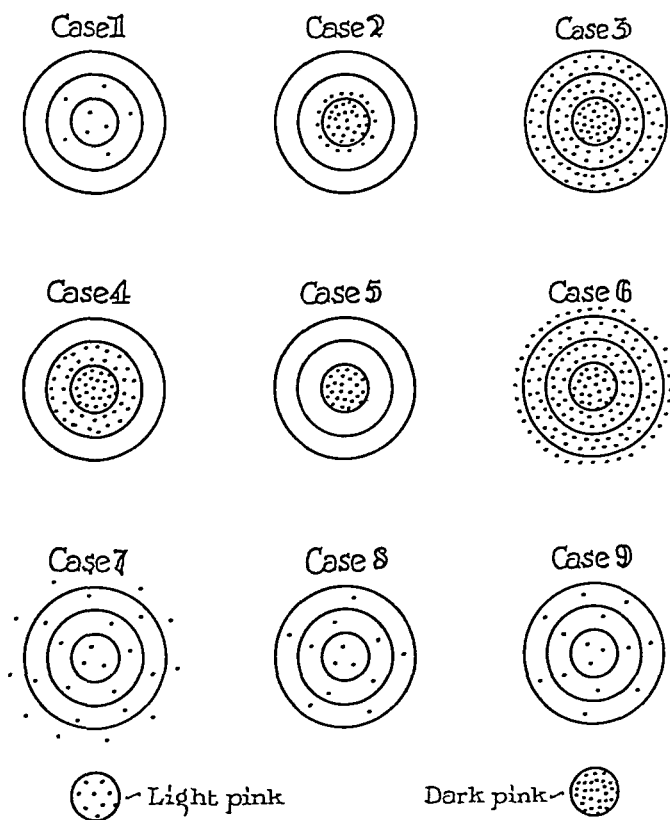


Fig. 4.

skin test interpretations, in a series of Schick and Dick tests, independent readings and recordings were made by two of us and when the final interpretations had been completed, a comparison of the two sets of records was made. Identical results were obtained in regard to the size of the reacting area. In regard to recording, the records tallied accurately, excepting in one or two instances where there was a difference of opinion as to whether the reacting area was light pink or dark pink. This did not alter the diagnosis of positive or negative but made a difference in the degree of positivity.

# SUMMARY

- 1 A simplified and practical method of measuring and recording skin tests has been presented
- 2 It has been used on adults and children with excellent results
- 3 In the hands of different individuals it produces strikingly similar results
- 4 While this method has only been employed in Schick and Dick testing it could be readily adapted to any form of skin testing

## CELL GROUP IDENTIFICATION OF DRIED BLOOD SPOTS AND TRACES OF SALIVA\*

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THE principles of inheritance of "blood" group specific factors are well worked out, and their forensic application in affiliation cases is receiving growing recognition.<sup>1 2 11 12</sup>

The subject of group identification of dried blood spots has received little recognition in this country. Landstamer in his first paper suggested the use of blood groups in the identification of human blood for medicolegal purposes, and with Richter, in 1903, experimentally demonstrated the feasibility of this suggestion.<sup>3</sup> This original work was based on the assumption that isoagglutinins are still present in dried blood and that autoagglutination is not a natural occurrence, hence, if an extract of a bloodstain is found to agglutinate the corpuscles of a given individual the stain cannot be due to this person's blood.<sup>4</sup> Since that time considerable work on this subject has been done in European countries. Through the initiative of Lattes<sup>5</sup> this work gained judicial recognition in Italy in 1916. Other case reports have come from Russia, France, and Germany.<sup>6</sup>

Mullei<sup>7</sup> extracted bloodstains with dilute saline solution and by vacuum distillation obtained a concentrated extract which was then tested with known cells for its agglutinin content. He claimed 70 per cent results with stains up to eighteen months old. Levine<sup>8</sup> reports the demonstration of agglutinins in a Group O stain four years old. Holzer found that after the first week attempts to demonstrate agglutinins gave clear cut results in only 50 per cent of the cases. Schiff and Higuchi used a test for agglutino-gen content based upon the absorption of agglutinins from a standard serum when exposed to dried blood. Holzer describes a quantitative modification of this test, he titrates the serum exposed to the bloodstain, and a portion of the serum exposed to a control portion of unstained material, using a capillary pipette to prepare progressively doubled dilutions on a special glass plate. Lattes<sup>9</sup> finds the quantities used by Holzer

\*From the Central Laboratories, Indiana University Medical Center.  
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suitable for measurement in graduated pipettes and titration in tubes; he recommends the use of absorbing serum in a dilution equal to one-sixteenth of its end-titer.

Jadin<sup>9</sup> has recently published results obtained by using the complement fixation test of Hirszfeld and Amzel.

Lattes<sup>8</sup> has pointed out that the conception of blood groups is rapidly becoming obsolete in the face of the newer conception of "cell groups" since group-specific factors (agglutinogens) are found in practically all cells (normal or pathologic), fluids and secretions of the body.

For further discussion of group-specific factors in body organs see Landsteiner and Levine<sup>10</sup> and Wiener.<sup>11</sup>

During the past year I have used various procedures suggested by European workers. By combining the extraction described by Jadin and the reconcentration suggested by Müller, and by testing for agglutinins and agglutinogens (agglutinin absorption and titration after the manner of Holzer), excellent results were obtained when adequate amounts of material were used. With marginal amounts the results were unsatisfactory. For the examination of small amounts of material a more sensitive technic was needed.

The following technic of agglutinin fixation was devised and has been found useful. The principle is the same previously employed but the amounts and methods afford higher sensitivity combined with greater speed and facility.

#### TECHNIC

*Demonstration of Agglutinogens.—Principle:* This demonstration depends upon specific fixation of agglutinins, contained in a standard serum, as a result of exposure to agglutinogens in the material being examined. This fixation is made apparent by finding upon subsequent titration that the titer of the test serum is reduced.

*Choice of a Standard Serum:* A Group 0 serum which has a strong titer approximately equal for A and B cells is required. Preliminary tests with 1:10 dilution upon slides will give a fair idea of suitability. If these tests seem satisfactory, the serum should then be titrated as follows:

- a. Prepare two series of progressively doubled dilutions (0.25 c.c. in 8 by 1.4 cm. test tubes).
- b. To each tube of the first series add 1 drop of an approximately 2 per cent suspension of A cells.
- c. To each tube of the second series add a similar portion of B cells.
- d. Shake to mix.
- e. Incubate five minutes at 20 to 25° C.; centrifuge three minutes at high speed.
- f. Shake to loosen the cells from the bottoms of the tubes and examine with a concave microscope mirror for agglutination.
- g. Record the findings and the end-titer for A and B cells.

*Agglutinin Fixation:* 0.2 sq. cm. of the stained cloth (conveniently measured as  $\frac{1}{8}$  of a disc 14 mm. in diameter) is placed in a test tube (8 by 1.4 cm.) and thoroughly wet by one drop of the standard Group 0 serum. The tube is incubated forty minutes at 37° C.

At the end of that time sufficient saline solution (0.85 per cent) is added to secure a dilution of the serum equivalent to one-sixteenth of its end-titer. For example, if the end-titer of the serum for A and B cells is 128, then a dilution of 1:8 is secured by the addition of seven drops of saline. The cloth is washed, pressed out, and removed from the tube by use of a glass rod.

Three drops of the resulting fluid are placed in each of two test tubes. To one of these (labeled AT) is added one drop of a 2 per cent (approximately) suspension of A cells, to the other (BT) a similar portion of B cells is added.

Simultaneously an equal amount of the cloth which is not stained is treated in exactly the same way. The tubes (AC and BC) containing the diluted serum which has been exposed to the unstained cloth serve as controls for the test proper.

The four tubes are incubated at 20 to 25° C for five minutes, then centrifuged at high speed for three minutes. They are removed from the centrifuge with care not to agitate them until they may be closely observed by means of a concave microscope mirror. AT and AC are shaken gently and the amount of agglutination in each is carefully compared, then BT and BC are examined.

*Comparative Titration.* If agglutination is present in both tubes, i.e. test and control the maneuver of comparative titration is employed. This consists of holding the two tubes in one hand and, with wrist motion only, shaking them gently at first then harder if necessary, to break up the clumps. The tubes should be shaken freely in the air so as not to strike against anything. The resistance of the clumps to shaking is proportional to the amount of agglutination and affords a comparative index of the titer of the fluid in the two tubes. If the clumps in the two tubes show an equal resistance to shaking, the titer of the test fluid has remained unchanged. If the clumps in the "T" tube disappear while the control still shows 2+ agglutination, it indicates a marked reduction in the titer of the test serum and the presence of the corresponding agglutinin in the stained cloth. Not infrequently when there is no reduction in titer in the "T" tube, it shows more agglutination than its control, this is probably due to the agglutinin content of the bloodstain which in this circumstance tends to increase rather than to decrease the titer.

In the absence of agglutination the cells usually float free with the slightest agitation. Occasionally they adhere to the bottom of the tube and require considerable shaking to loosen, whenever they loosen they float free without clumps. This difficulty is usually the result of a light cell suspension.

Recentrifugalization and rereading are frequently helpful if the first reading is not clear cut. Microscopic readings are essentially noninforming after one has gained some experience with the concave mirror.

*Demonstration of Agglutinins.*—The technique used here for this purpose differs in no essential way from that used by Lattes and others.

A portion of the stained material is extracted with the smallest possible amount of 0.25 per cent saline solution. A drop of the extract is placed on each of three cover slips and allowed to evaporate to dryness. Second and third drops may be superimposed.

To one of these is added a drop of a suspension of A cells (approximately 2 per cent) sufficiently small that it is contained within the rim of the dried extract. The cover slip is inverted and placed upon a hanging drop slide. B cells are added to the second cover slip and O cells to the third.

The slides are incubated at 20 to 25° C. Final readings should not be taken before eight or twelve hours.

Agglutination of 1+ or more with both A and B cells indicates Group O, agglutination with only the B cells indicates Group A, agglutination only with the A cells indicates Group B. The failure to observe agglutination is indicative of Group AB, but this is of little significance unless the stain is known to be less than ten days old. The O cells serve as a control against pseudo and hetero agglutination.

## EXPERIMENTAL RESULTS

To determine the efficacy of these methods under unknown conditions, blood and saliva stains were prepared in the hospital admitting room and sent to the laboratory labeled only by serial number. The bloodstains upon freshly laundered white shirt cloth were sealed in envelopes by the various individuals

supplying the blood, thus securing the saliva specimens. Grouping tests upon fresh cell suspensions were made by another worker who withheld results until completion of the tests on the stains. The results of these examinations are recorded in Table I.

TABLE I

## DIAGNOSES OF UNKNOWN SPECIMENS FROM THIRTY INDIVIDUALS

Specimens 2-3 weeks old when tested. Figures based on single examination. Saliva specimens taken from gummed flaps of the envelopes.

SPEC. NO.	BLOODSTAINS		FINAL DIAGNOSIS	SALIVA STAINS	
	AGGLUTININ CONTENT	AGGLUTININ FIXATION		AGGLUTININ FIXATION	KNOWN GROUP
1	?	O	O	O	O
2	O?	O	O	O	O
3	O?	O	O	O	O
4	AB?	O	O	O	O
5	A	A	A	A	A
6	O?	O?	O	O	O
7	A	O*	A	O*	A
8	O?	O	O	O	O
9	O	O	O	O	O
10	O	O	O	O	O
11	A	B*	?	A	A
12	A	A?	A	A	A
13	?	O	O	O	O
14	B	B	B	O*	B
15	O	O	O	O	O
16	A	O*	A	A	A
17	O?	O	O	O	O
18	A	O*	A	A	A
19	?	A?	A?	A	A
20	AB, O?	AB, O?	AB?	O*	AB
21	O	O	O	O	O
22	O	O	O	O	O
23	?	A, O?	?	A, O?	O
24	O	O, B?	?	O	O
25	A, O?	O	?	O	O
26	O?	O	O	O	O
27	O	O	O	O	O
28	O	O	O	O	O
29	O?	O	O	O	O
30	O	O?	O	O	O

Correct 16 (53.3%) 19 (63.3%) 24 (80.0%) 26 (86.7%)  
 inconclusive 14 (46.7%) 7 (23.4%) 6 (20.0%) 1 (3.3%)  
 \*Misdiagnosed 0 (0.0%) 4 (13.3%) 0 (0.0%) 3 (10.0%)

On the basis of a single examination of the thirty bloodstains, 16 (53.3 per cent) were correctly diagnosed by testing for agglutinin content. In 14 (46.7 per cent) the results were inconclusive.

By means of the agglutinin fixation test, 19 specimens (63.3 per cent) were correctly grouped. Seven (23.4 per cent) did not give conclusive results. Four (13.3 per cent) were misdiagnosed because of false positives. The correct grouping of each was the positive reaction. The correct grouping of each was the positive reaction.

By evaluation of the findings given to positive evidence (80 per cent), while in 6 cases none were misdiagnosed. Specimens were identified as Group

Of the 30 saliva specimens, 26 (86.7 per cent) were correctly grouped by the agglutinin fixation test. One specimen (3.3 per cent) was not definitely diagnosed. Three (10 per cent) were missed because of false negative reactions.

To determine the sensitivity of this technique of agglutinin fixation in terms of milligrams of dried blood, ten  $\frac{1}{8}$  discs of bloodstained cloth from various of the specimens used above were weighed. The weight of ten identical portions of unstained cloth was subtracted, the difference divided by 10 gave 0.84 mg of dried blood per  $\frac{1}{8}$  disc or per 0.2 sq. cm.

A set of known stains showed no decrease in the activity of agglutinogens when last tested by this method at the end of five months.

Through the kindness of Dr. Karl Landsteiner in providing samples of anti-M and anti-N testing fluid, it has been possible to select individuals of type MN, M, and N. From preliminary tests with prepared stains, it appears that this technique is also adaptable to the demonstration of M factor.

#### DISCUSSION

*Discussion of Technique*—In testing for agglutinins the leucithin suspensions recommended by Lattes have caused a slight desensitization of isoagglutination without better control of pseudoagglutination than is afforded by the use of O cells. Pseudoagglutination is not a problem in the agglutinin fixation test because of the dilution of the serum.

Lattes recommended the use of absorbing serum in a dilution equivalent to one sixteenth of its end titer. I believe that by exposing the stained material to an appropriate dose of undiluted serum a more intimate contact between agglutinins and agglutinogens and consequently a more thorough and rapid fixation is secured. The subsequent dilution to one sixteenth of the definitive titer procures not only a working volume but also the optimum sensitivity for avoiding both nonspecific fixation and the masking of slight specific fixation which Lattes feared.

The centrifuge test method for demonstrating hemagglutination has been derived from Wiener and Vaisberg<sup>13</sup> to whom it was suggested by Landsteiner and Levine. Similar methods had previously been described by Levine and Mabee<sup>14</sup> and by Schiff.<sup>15</sup> The quantitative relationship between the resistance of the clumps to shaking and the titer of the test fluid has not been emphasized previously.

The technique described above for demonstrating agglutinogens is characterized by certain desirable features, namely:

a. It requires only about one tenth the amount of material needed for most previous techniques of agglutinin fixation or absorption.

b. The handling of materials is minimum, thus reducing the probability of technical error.

c. The speed makes for greater facility and for less bacterial interference.

The question of just what reduction in titer is of diagnostic significance is best answered by personal experience with the test. I regard a difference of

two grades (as C:2+; T:tr) as definitely diagnostic. However, in unchanged fluid the agglutination is surprisingly constant and a one-grade decrease in titer should be regarded with suspicion and rechecked.

In the experimental work, cells from various individuals have been preserved in Rous-Turner solution and washed in saline before use. For actual case work the cells and serum should be chosen from individuals of known group whom experience has shown to be free from atypical agglutination characteristics, and who will be available later if needed. The cells should be fresh and washed once in saline. Lattes recommends that the serum be separated at 0° C. to free it from cold agglutinins. Schiff prefers a mixture of serums of groups A and B adjusted to an equal titer for both factors. No serious difficulty has been experienced here in securing satisfactory Group 0 serums which do not require adjustment.

*Discussion of Results.*—In considering the experimental results it should be remembered that this technic is primarily designed for the examination of minimal stains unsuitable for the less sensitive method; that the figures are based upon a single examination; and that the attempt to demonstrate agglutinogens might have been several times repeated, still using less material than the first method mentioned.

Particular attention is called to the diagnoses obtained by correlating the findings of agglutinin and agglutinogen demonstrations. The greater percentage of correct diagnoses and the absence of false diagnoses leave no doubt of the desirability of attempting both demonstrations.

The results indicate that this technic is particularly suitable for demonstrating agglutinogens in saliva dried upon paper. The false negatives in the above series may be due to nonsecretion types; this has not been investigated.

Previously the demonstration of agglutinogens has been a second choice to the demonstration of agglutinins because it required a greater amount of material. Various technics require from 10 to 50 mg. of dried blood which can be lifted as a crust from the substratum, or at least 2 sq. cm. of bloodstained cloth. The test for agglutinin content is reported to be sensitive to 0.1 mg. of dried blood (crust?).

In using bloodstained cloth and the technic described above, I find that the demonstration of agglutinins requires more material than does agglutinogen demonstration. Moreover, the reliability of the test for agglutinins is inversely proportional to the age of the bloodstain, while the test for agglutinogens is not so affected.

*General Discussion.*—In this investigation interest has been centered upon the demonstration of agglutinogens because: these factors are more resistant to deterioration from age and physical exposure; they are found in body cells and secretions where the agglutinin content is low and inconstant; and finally only by such an approach will it be possible to demonstrate the factors M and N of Landsteiner and Levine.

The greatest objection to this test is that, since such a great part of our population belongs to Group 0, it will show only negative evidence in nearly half of the stains examined. Some workers have stated that a diagnosis of Group 0 should never be made by agglutinin fixation unless either M or N

agglutinin is demonstrated. This is desirable but not always practical. A positive anti human precipitin test is prerequisite to the grouping tests. In the presence of a positive precipitin test negative evidence for A and B cells should be evaluated in the light of a definite history of the accident and physical exposure of the stain. In favorable instances it seems reasonable to diagnose Group O without demonstrating either M or N factors. Repeated tests could always be made if the material at hand permits.

In forensic work the method of attack must be suited to the individual case after consideration of the legal problem as well as the technical available. The theoretical limitations of blood grouping in solving the legal problem and the technical limitations in solving the scientific problem make conservatism imperative. If this attitude is maintained by workers undertaking forensic investigations, there is every reason to believe that such investigations may be in the future an invaluable aid in the administration of justice.

#### SUMMARY

A method for demonstrating group specific substances in dried blood, by extracting with dilute saline, reconcentrating the extract and testing for agglutinins and agglutinogens after the manner of various European workers, gave satisfactory results in the hands of the investigator only when a sufficient quantity of material was available. Group identification of minimal amounts of bloodstained material by this method was time consuming and uncertain.

A technique of agglutinin fixation sensitive to 0.84 mg of dried blood is described. The test is made with a small amount of Group O serum allowing a forty minute period for fixation. The serum is then diluted to one sixteenth of its definitive titer and tests for fixation of the agglutinins are made by using A and B cells. For these tests the centrifuge test method is used in such a way that partial fixation of the agglutinins may be demonstrated and quantitatively estimated. The time required for this determination is about one hour as contrasted to twenty four to forty eight hours in the previous technique.

A technique for the demonstration of agglutinins in dried blood is described.

The results of examination by these methods of unknown blood and saliva stains from thirty individuals are presented.

While deterioration with age and exposure presents some difficulties in demonstrating agglutinins the desirability of attempting to do so is pointed out.

Appreciation is expressed for the invaluable suggestions and criticism of Dr C G Culbertson, for the advice and constant encouragement of Dr R N Harger, for the assistance of Dr Phillip Kurtz in translation of the German references, and for the co-operation of fellow workers in the preparation of unknown specimens and in the direct group determinations.

#### REFERENCES

1. Heise, H. A. Some Medicolegal Aspects of Isoagglutinins, *Am J Clin Path* 4: 400, 1934.
2. Levine, P. Blood Groups, Theory and Medicolegal Application, *J Lab & Clin Med* 20: 785, 1935.
3. Jansser, Hans, and Coca, A. F. Remarks Concerning Landsteiner's Discovery of Isoagglutination and Blood Groups, *J Immunol* 20: 259, 1911.

4. Lattes, L.: *Individuality of the Blood*, Translated by L. W. H. Bertie, 1932, Oxford University Press.
5. Muller, M. A.: *Das Agglutinin-anreicherungsverfahren*, Deutsche Ztschr. f. d. ges. ger. Med. 11: 120, 1928.
6. Levine, P.: *The Application of Blood Groups in Forensic Medicine*, Am. J. Police Sc. 3: 166, 1932.
7. Holzer, F. J.: *Ein einfaches Verfahren zur Gruppenbestimmung an Vertrocknetem Blut durch Agglutininbindung*, Deutsche Ztschr. f. d. ges. ger. Med. 16: 445, 1931.
8. Lattes, L.: *Les Groupes Sanguins en Medicine Legale*, Ann. Med. Leg. 14: 245, 1934.
9. Jadin, J.: *L'identification des taches de sang*, Arch. Internat. de Med. Exper. 9: 325, 1934.
10. Landsteiner, K., and Levine, P.: *On Group Specific Substances in Human Spermatozoa*, J. Immunol. 12: 415, 1926.
11. Wiener, A. S.: *Blood Groups and Blood Transfusion*, Springfield, 1935, Chas. C. Thomas.
12. Levine, P.: *Wisconsin Law on Blood Tests*, J. A. M. A. 105: 1370, 1935.
13. Wiener, A. S., and Vaisberg, M.: *Heredity of Agglutinogens M and N*, J. Immunol. 20: 371, 1931.
14. Levine, P., and Mabee, J.: *A Dangerous "Universal Donor,"* J. Immunol. 8: 425, 1923.
15. Schiff, F., quoted by: Levine,<sup>6</sup> Lattes,<sup>4</sup> Lattes,<sup>8</sup> and Wiener,<sup>11</sup>  
Schiff and Higuchi, quoted by: Holzer.<sup>7</sup>

## A NEW RAPID METHOD FOR FROZEN SECTION DIAGNOSIS\*

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THE method employed in this laboratory and in most institutions for the quick diagnosis of tissue biopsies has been that which has been described and modified by Broders, Terry, and others at various intervals during the last decade. This technic consists of floating the frozen section out on a solution of Terry's polychrome methylene blue, washing in water, and then mounting the stained section on a slide by means of a glass rod and dropping on a cover slip. The microscopic picture then presented shows excellent cellular detail and some color differentiation of the various types of tissue elements, and in the hands of an experienced tissue technician the results are uniformly good. One difficulty that is occasionally encountered is the presence of fat in certain blocks, such as in a breast tumor or axillary glands, where the fat globules obscure the picture and apparently alter its staining properties. Another difficulty is experienced in smaller institutions where the technician does not devote sufficient time to sectioning alone to become technically proficient and his results may be so infrequent as to afford but little practice.

A method devised in this laboratory using the above polychrome methylene blue stain is more rapid, simpler, and more accurate.

This method in brief is as follows: The tissue is frozen in the freezing microtome in the usual manner; the slide; the excess of water is removed; the slide is taken not to touch the water.

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## THE DERIVATION OF AN INDEX NUMBER FOR THE OPSONOCYTOPHAGIC TEST\*

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THE opsonocytophagic test devised by Huddleson Johnson and Hamann<sup>1</sup> for assaying the clinical status of patients in relation to brucellosis is gaining widespread use. A recently published confirmation<sup>2</sup> of the original author's findings will doubtless increase the demands for this test. In addition to its diagnostic value the test is extremely useful in estimating the therapeutic efficacy of various types of treatment, especially with specific vaccines, filtrates and antiserums. We have also found the procedure valuable as a measure of the development of potency of antibrucella and antitularensis serums when monthly trial bleedings are made from animals undergoing prolonged series of inoculations.

When evidences of increasing immunity were being sought in patients under treatment or in animals under inoculations, it became evident to us that a convenient and accurate index number for each of the serial counts would be very advantageous. An index number is much more conducive to a rapid understanding of the value of the test and the meaning of its results than a presentation of the usual four columns of figures. The following method for the derivation of such an index number is offered to fill this need.

If the occurrence of phagocytosed bacteria be set up in four classes, namely those cells showing no inclusion of bacteria, those showing from 1 to 20, those from 21 to 40 and a final class containing 41 and over, observation and counting of a given smear will result in a frequency distribution whose frequencies are distributed in four arrays under the above rubrics. The aim of the index number is to summarize these four entries into a single number that bears some easily translatable relationship to the frequencies as they fall into the respective arrays. And further, the index must recognize that the arrays are not equal in significance. A cell showing no inclusions, and by the same token a cell showing a large number of inclusions, are both of greater biologic significance than cells showing moderate degrees of phagocytosis. Therefore we decided to weigh the extremes as against the two intermediate arrays. This was accomplished by placing the arbitrary origin between the Groups 1 to 20 and 21 to 40. In order to avoid the disappearance of frequencies due to multiplication by a zero moment the first group on either side of the origin was assigned a moment arm of one, the sign being positive to the right and negative to the left. The next moment was assigned the value of two, and again positive to the right and negative to the left. This value is obviously an arbitrary one and implies that the

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two extremes are biologically twice as significant as the two arrays next to the point of origin. We do not know this to be true nor could we say that the extremes are of equal significance. But for the sake of simplicity it seemed advisable to use this arrangement. It would seem of little importance what values are assigned to the moment arms as long as the structure of the index number is clearly understood.

With the values described above it is apparent that a given set of four frequencies, each multiplied by the respective moment arm, may be summarized, with due regard to algebraic sign, into a single number which is bound to fall between  $-100$  and  $+100$  as the two maximum values, with the former indicating no inclusions and the latter the highest degree of inclusion. Zero would represent the situation midway between these two extremes. To avoid the signs we decided to refer the range  $-100$  to  $+100$  to a new base entirely positive in sign and having a range from 0 to 100. Thus 0 on this final scale is equal to  $-100$  on the original, 50 is equal to 0, and 100 is equal to  $+100$ .

For example, a count recently made on a patient at the end of ten days of vaccine therapy fell as follows:

NO BACTERIA	1-20	21-40	41 OR MORE
12	18	6	14
	-	+	
$\times 2$	$\times 1$	$\times 1$	$\times 2$
-24	-18	+6	+28

The sum of the four products is  $-8$  in the  $-100$  to  $+100$  range, corresponding to 46 on the final base. The phagocytic index number is therefore 46. The count taken twelve days previously showed:

NO BACTERIA	1-20	21-40	41 OR MORE
24	16	9	1
	-	+	
$\times 2$	$\times 1$	$\times 1$	$\times 2$
-48	-16	+9	+2

The sum of the products was  $-53$ , corresponding to a phagocytic index number of 23.

A patient with severe acute brucellosis acquired by grinding condemned meat in a local packing house was treated with antibrucella serum in the wards of the Cincinnati General Hospital. Porcine strains were recovered from each of three blood cultures. Serum was administered immediately after the first phagocytic test was performed. The serial counts, most made by Mr. A. E. O'Neill, and their computed index numbers were:

DATE	NO BACTERIA	1-20	21-40	41 PLUS	P. I. N.
8/12/35	35	10	1	4	15
8/15	34	14	0	0	9
8/20	11	27	10	2	33
8/24	10	32	8	0	28
8/30	17	22	8	3	29
9/ 4	24	22	4	0	17
9/10	10	22	15	3	40
9/17	0	8	34	8	71
9/22	0	7	17	26	81
10/ 5	0	6	10	34	86

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We have computed several hundreds of actual and hypothetical counts to test the reasonableness of the derived phagocytic index number against the actual distribution of cells in the four customary rubrics. In each instance the 'fit' was satisfactory and the phagocytic index number as obtained was considered an appropriate and reasonably accurate expression of the immediate immune status of the patient or animal.

Our interest in counting a total of 50 cells in each film was originally prompted by curiosity to see how closely the distribution of a second 25 cells would follow that of the first. We have habitually recorded our counts as two series of 25 cells each, one under the other. In one case the count was recorded as follows:

NO. BACTERIA	1-20	21-40	41 Or More
10	10	4	1
(	7	8	4

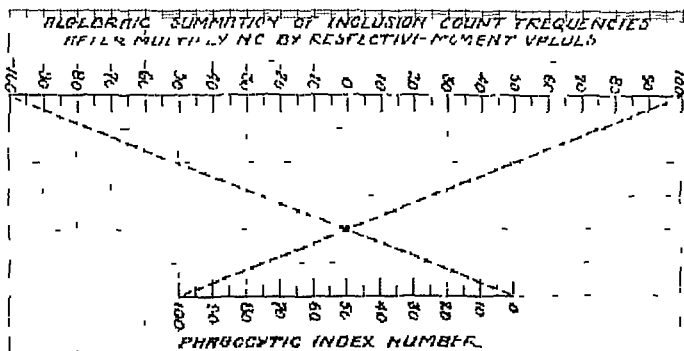


Fig 1—Nomogram for the conversion of inclusion counts into phagocytic index numbers. The algebraic sum of the weighted inclusion count frequencies is located on the 100 to +100 scale. A straight line from this point passing through the central black dot intersects the smaller scale at a point where the phagocytic index number may be read off directly.

In order to make a 25 cell count fit into the scheme of index number derivation outlined above, it is evident that such count figures must be doubled in one of two ways. Either the recorded figures themselves are doubled and then multiplied by the usual moment factors of 1 and 2 in the usual way, or the recorded figures are taken as such and are multiplied by the doubled moment factors, 2 and 4, instead of the usual 1 and 2. The resultant sum of the products will, of course, be the same in either case. If the above 50 cell count is computed in the usual way the phagocytic index number will be 36. However, if the two 25 cell counts are computed separately, the resultant index numbers will be 26 and 47, respectively. The average of these numbers is 36.5 but the spread of 21 points between them is high, comparable to an error of approximately  $\pm 10.0$ . When the distributions of the two 25 cell counts are very similar, as in the first

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example cited, the error is of course very small. In this count the actual figures were:

NO BACTERIA	1-20	21-40	41 OR MORE
6	9	2	8
6	9	4	6

On the basis of a 50 cell count the phagocytic index number was 46. If each set of 25 cells is computed separately the index numbers are 47 and 45, respectively, a divergence of only  $\pm 1.0$ . It is believed that index numbers of greater accuracy and reasonableness are obtained from counts of 50 cells than from counts of 25 cells. In experienced hands 25 cell counts are probably satisfactory.

The accompanying nomogram (Fig. 1) furnishes a convenient means for translating the summed products from the -100 to +100 range to the 0 to 100 scale.

In practice one can take the index number as a percentile expression of the immediate capacity of the patient to phagocytose bacteria. Thus, in the count next above the patient's blood possessed 46 per cent of his total potential capacity to phagocytose brucella.

#### REFERENCES

1. Huddleson, I. F., Johnson, H. W., and Hamann, E. E.: A Study of the Opsono-Cytophagic Power of the Blood and Allergic Skin Reaction in Brucella Infection and Immunity in Man, *Am. J. Pub. Health* 23: 917, 1933.
2. Keller, A. E., Pharris, C., and Gaub, W. H.: Diagnosis of Undulant Fever. The Opsono-cytophagic, Allergic and Agglutination Reactions, *J. A. M. A.* 107: 1369, 1936.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE, M D, ABSTRACT EDITOR

## SPIROCHETAL JAUNDICE, Serum Diagnosis by Precipitation, Eber, B C R Soc Biol 120 618, 1935

The following is the technique used at the Pasteur Institute, Paris

A four day old culture of the living spirochete in 20 per cent rabbit serum in saline was generally used, and it is necessary to examine it by the dark ground to see that active spirochetes are present If older cultures are used, they should be centrifuged for a short time to remove any debris from the medium The patient's serum should be fresh and clear and when examining cerebrospinal fluid no traces of blood should be present

For the test three tubes are used the first receives 0.1 cc of the undiluted serum, the second 0.1 cc of the serum diluted 1:10 and the third 0.1 cc diluted 1:100, 0.9 cc of culture is added to each of the tubes and also to a fourth tube, which receives 0.1 cc of saline and serves as a control The tubes are kept at 37° C for one hour before examination With cerebrospinal fluid, dilutions of 1:10, 1:50, and 1:100 are used As a rule this test is sufficient to indicate whether the specimen is positive or negative, but in some cases the titer of the serum is of interest and then dilutions up to 1:100,000 or more are prepared

Examination is always made by means of dark ground illumination using a No 6 objective and No 4 eyepiece With positive sera, dilutions of 1:10 generally contain free spirochetes, but their movements are feeble, in addition there are a few spirochetes agglutinated in irregular masses In the 1:100 dilutions, there are free spirochetes and also more or less spherical masses covered with feebly motile spirochetes undergoing lysis In the 1:1,000 dilution, there are numerous small masses of agglutinating spirochetes somewhat resembling the appearance of red blood cells in a hypertonic solution, usually no free spirochetes are present If the spirochetes remain active, even if they agglutinate in the lower dilution, the reaction is doubtful and if a second test with serum collected a few days later gives similar results, it must be regarded as negative

Agglutination may be observed commonly in dilutions up to 1:100,000, but lysis soon disappears in dilutions above 1:1,000

With cerebrospinal fluid doubtful reactions have not been observed since the limit of dilution rarely exceeds 1:100, even when the serum of the patient agglutinates in dilutions of 1:100,000

## TUBERCLE BACILLI Studies on the Dissociation of, With Special Reference to the Avian and Human Types, Alexander Jackson, E Am Rev Tuberc 33 767 1936

Three strains of human tubercle bacilli have been completely dissociated from R to S

Human type S colonies are convex, round, smooth edged and glistening and resemble the S colonies of bovine and avian tubercle bacilli

The addition of small optimal amounts of ferric chloride 0.0004 per cent, to Bordet Gengou medium favored the formation of S colonies of the human type in pure culture

Lack of sufficient blood in Bordet Gengou medium, and the addition of crystal violet to Long's medium are inhibitory to the formation of S colonies Crystal violet in 0.01 per cent amounts discouraged avian S, and a concentration of 0.001 to 0.0001 per cent sufficed to inhibit the human type S

The lesions produced by avian and by human R bacilli tended to be limited, calcified, and macroscopic, and contained relatively few bacilli, whereas those produced by the S

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fluid decanted into the volumetric flask, the flask shaken to insure mixing of the contents, and placed in a bath of boiling water for 5 minutes, at the end of which time it was removed and cooled in running water; when thoroughly cooled, 1 c.c. of 2 per cent  $\text{NaNO}_2$  was added, the mixture carefully shaken and allowed to stand for 2 minutes, at the end of which time the readings were made. In order to facilitate the detection of the pink coloration, indicating a positive test, the flasks were observed against a white background, using a similar flask containing distilled water as a control. The amount of free tyrosin detected in each of the positive tests was insufficient to be quantitated colorimetrically, hence designations of strongly positive (+++), positive (++), faint (+), or negative were utilized, according to the intensity of the pink coloration present in the final solution after the addition of the sodium nitrite.

**TUBERCULOSIS, The Filament-Nonfilament Count in Tuberculosis Compared With the Sedimentation Rate and the Leukocytic Index, Paine, D., and Austin, K. H. Am. Rev. Tuberc. 35: 221, 1937.**

The nonfilament count and leukocytic index were remarkably often in agreement. Certainly the nonfilament count in this study proved no more delicate than the leukocytic formula as an index of coming trouble. The sedimentation rate, as other observers have noted, sometimes remains high after the nonfilament count and the leukocytic index have returned to normal and the patient has become clinically inactive. The personal equation is a factor in doing nonfilament counts because many cells are hard to classify. This, however, is true also of the leukocytic index where the differentiation between monocytes and large lymphocytes is often difficult.

Filament-nonfilament counts done on 50 normal people varied from 2 to 16 per cent, averaging 7.7 per cent.

Filament-nonfilament counts, leukocytic counts, and sedimentation tests were taken simultaneously at intervals on 77 tuberculous patients.

In only 9 of the patients studied were the trends of the three determinations in disagreement. For the most part filament-nonfilament and leukocytic counts agreed closely.

In the 16 patients showing steady progression of the lung process, all three tests were elevated consistently.

In 4 patients showing a sudden and marked increase of disease neither filament-nonfilament nor leukocytic index showed any premonitory rise.

Sedimentation rates remained elevated in some patients who were clinically inactive and whose leukocytic index and nonfilament count had returned to normal.

The filament-nonfilament count in this study demonstrated no superiority over the leukocytic index.

**TUBERCULOSIS, The Autourine Test in the Diagnosis of, Hanan, E. B., and Zurett, S. Am. Rev. Tuberc. 35: 229, 1937.**

It is suggested that more careful studies on the chemical and biological nature of the antigenic substances excreted in the urine from individuals with infectious diseases would increase our understanding of the immunology and pathology of these diseases.

The technique for the administration of the autourine test for tuberculosis and the factors involved in the interpretation of the skin reactions are described.

Two types of autourine skin reactions are described, and theoretical considerations concerning the specificity of the tuberculous urinary antigen are discussed.

The Enright-Rettger modification of the Wildholz autourine test for tuberculosis was applied to 147 cases of known and suspected tuberculosis, with 92 per cent agreement with the final clinical and laboratory analysis.

A simplified procedure for the autourine test, using a mixture of ether and chloroform for extraction of the tuberculous antigen, thus eliminating the complicated steps of concentration and dialyzation of the urine, is described.

The ether-chloroform autourine test for tuberculosis was applied to 206 cases. The test was positive in 87 per cent of 45 of the cases known to have active tuberculosis. In 63

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borderline cases the test was positive for active tuberculosis in 34 and negative in 29. In 80 cases of disease other than tuberculosis, the test was positive for tuberculous activity in 1 and negative in 67. The test was negative in 13 normal individuals and positive in 5 cases of bone and joint tuberculosis.

The method follows:

**Technique of the ether-chloroform autourine test.** The procedure employed was as follows: 150 cc of freshly collected morning urine were made slightly alkaline to litmus with sodium bicarbonate. Fifty cubic centimeters of ether and chloroform in equal amounts were added and the mixture shaken vigorously in a mechanical shaker for 10 minutes. The emulsion was placed in a separatory funnel and when the separation appeared the extractives were run into a dish and evaporated just to dryness on a water bath. The residue was suspended in 10 cc of sterile salt solution. Approximately 0.1 cc or a sufficient amount to produce a 3 mm skin bleb, was used for the test.

**STREPTOCOCCIC INFECTIONS. Method of Determination of Para aminobenzenesulfonamide (Prontosil) in Urine and Blood, Marshall, E. K., Emerson, K., Jr., and Cutting, W. C. J. A. M. A. 108 923 1937**

A method has been devised for determining para aminobenzenesulfonamide in blood and urine. A conjugated form excreted in urine can be determined after hydrolysis with dilute hydrochloric acid.

In the dog, the substances appear to be excreted unchanged in the urine, while in the rabbit large amounts are excreted in a conjugated form. In man, the substance is excreted in both the free and the conjugated forms.

Absorption from the gastrointestinal tract is rapid, being usually complete or nearly complete in about four hours.

In dogs, the concentration in the blood does not mount more quickly or attain a higher level with subcutaneous administration than with oral.

In patients, when large amounts are administered daily in divided doses, nearly 100 per cent may be recovered from the urine when equilibrium between intake and output is established. It takes from two to three days to establish this equilibrium and the same time to free the body of the drug after it is discontinued.

In patients with impaired renal function, the sulfonamide appears to be excreted more slowly. Until more data are available it should be given with care in all cases of renal insufficiency.

After oral administration, the sulfonamide is found to be present in the cerebrospinal fluid in a somewhat lower concentration than in blood.

The method follows:

For the determination of para aminobenzenesulfonamide in blood and urine, the following reagents are necessary:

- 1 Tenth normal hydrochloric acid
- 2 Sodium nitrite 0.1 per cent (freshly prepared)
- 3 Ethyl alcohol (95 per cent)
- 4 Dimethylanaphthylamine, 1 cc to 100 cc of alcohol

5 A standard solution of para aminobenzenesulfonamide, 200 mg per liter. From this solution standard solutions containing 10, 0.5 and 0.2 mg per hundred cubic centimeters can be prepared. The standard solution appears to keep unchanged for several months if kept in the icebox.

Urine is diluted so that the diluted solution contains from 0.5 to 1.5 mg per hundred cubic centimeters of sulfonamide. Ten cubic centimeters of this diluted urine is measured into a small flask and 2 cc of hydrochloric acid, 1 cc of sodium nitrite, 5 cc of alcohol and 1 cc of dimethylanaphthylamine are successively added. The flask is shaken after the addition of each reagent. Ten cubic centimeters of an appropriate standard is similarly treated. After a few minutes standing, the solutions are compared in the colorimeter. Just after the dimethylanaphthylamine has been added the solutions may not match exactly while

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after too long standing an orange tint occurs in the diluted urine which gives low readings. Readings are usually taken from five to ten minutes after the addition of the reagents. The color developed in standard solutions in pure water appears to remain unchanged for several hours.

For blood, the following procedure is used: One volume of blood is run slowly with shaking into 9 volumes of alcohol, and the flask is stoppered and allowed to stand ten minutes or longer. The mixture is now filtered and 10 c.c. of the filtrate measured into a small flask. Five cubic centimeters of water, 2 c.c. of hydrochloric acid, 1 c.c. of sodium nitrite, and 1 c.c. of dimethylanaphthylamine are successively added. The colored solution is slightly turbid but after standing five minutes can be filtered and the clear filtrate used for colorimetric comparison. An appropriate standard is prepared at the same time by adding 1 c.c. of a standard solution of the sulfonamide to 9 c.c. of alcohol and treating this solution as described for the blood filtrate. Color comparison is best made about fifteen or twenty minutes after the reagents have been added. Since only 92 per cent of the sulfonamide is recovered from blood by this procedure, the final result is divided by 0.92 to obtain the correct concentration in blood. Subsequently it was found that if the dimethylanaphthylamine is added about three minutes after the sodium nitrite a more intense color is obtained and the recovery is practically 100 per cent.

The accuracy of this method has been checked on pure solutions of sulfonamide, on normal urine, and on urines containing sulfonamide to which more sulfonamide has been added, and on normal dog and human blood after the addition of sulfonamide. Duplicate determinations usually check within 2 or 3 per cent.

In the rabbit and the human subject, this compound is partly excreted in the urine as a conjugated compound which does not give the color reaction directly, owing to a blocking of the amino group. The para-aminobenzenesulfonamide can be obtained from this compound by hydrolysis with dilute hydrochloric acid. To determine this conjugated compound in urine, heat equal volumes (usually 1 c.c.) of urine and normal hydrochloric acid in a test tube (25 by 200 mm.) covered by a small beaker in boiling water for thirty minutes. The solution is cooled and, after the addition of 1 drop of 0.1 per cent phenolphthalein, neutralized with 2 normal sodium hydroxide. After dilution to appropriate volume, the determination is performed as described for urine. Samples of urine heated with 1 or 3 normal hydrochloric acid for thirty, forty-five and sixty minutes yield identical values for the conjugated compound, and subjecting a solution of sulfonamide of known strength to acid hydrolysis results in no loss as determined by the colorimetric method. This would indicate that the method is accurate for determining the hydrolyzable material in urine.

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erythyl blue (Coleman and Bell) in normal saline solution is then dropped on the center of the section which stains it instantly. A cover slip is then placed on the stained tissue and the slide given a gentle tap downward on the finger to fix the cover slip in position, and the tissue is ready for examination.

It will be noted that the dye colors the entire slide with the exception of the tissue, and it possesses a different color further than the pale bluish halo immediately surrounding the section. This phenomenon prevents the possibility of a dark background which one might expect and avoid, without the staining a superfluous step.

Although the polychrome method is sufficiently rapid for all emergency surgical procedures the method just described is even more so, none being that staining is instantaneous and that the time required for washing is eliminated. A very definite advantage is the sureness with which good results are obtained, it being virtually impossible to over- or understain a tissue, and there is almost complete freedom from fat globules and air bubbles. The prepared sections for some obscure reason appear somewhat more resistant to drying out. The color differentiation is very similar to that of Terry's polychrome methylene blue, the fibrous or connective tissue assuming a reddish or purple tint, while the parenchymatous cells, mucus, etc., take varying shades from blue to green. The intracellular structure, however, appears somewhat different with the two dyes, the entire cell structure staining better with the Terry method, while the nuclear components appear more distinct with the vital stain.

I have often had occasion to cut sections on formalin fixed tissue from rapid diagnosis and have found this method very satisfactory, if polychrome stain were used on this tissue it would tend to overstain. In some types of unfixed tissue we have used 1 per cent solutions of brilliant erythyl blue, but in our experimental work solutions more dilute than 1/200 (0.5 per cent) are unsatisfactory. The solution does not seem to deteriorate and I have found that a fine tipped medicine dropper placed in the stopper of the bottle is a convenient way of dispensing it.

#### SUMMARY

A method is presented for staining in rapid tissue diagnosis which employs a vital stain—brilliant erythyl blue, it is simpler, more rapid, and more satisfactory in inexperienced hands than some methods in vogue.

# A TECHNIC FOR PERFORMING A VALVULAR CECOSTOMY IN THE DOG\*

GEORGE R. COWGILL, PH.D., AND LOUIS WEINSTEIN, PH.D., NEW HAVEN, CONN.

THE value of animals possessing a fistula in the large intestine at the level of the cecum is at once apparent to the investigator whose chief interest lies in the physiology and bacteriology of the colon. This type of opening has been made in dogs before, but leakage of intestinal contents has given difficulty. Animals with leaking fistulas are not easily kept in a good state of nutrition because of loss of water and salts, and also because of erosion of the body wall due to more or less continual contact with the intestinal discharge. This paper describes a cecostomy technic which includes the making of a valve which either eliminates leakage entirely, or reduces it to a point which permits a long normal life and obviates the necessity of continual bathing, bandaging, and nursing care.

## PROCEDURE

Female dogs weighing from 12 to 15 kilos are treated with a vermifuge about one week previous to the operation. Preoperative treatment further consists in withholding food for forty-eight hours and allowing water *ad libitum*. We prefer to use morphine and ether as the anesthetics in our operations.

Although the cecum of the dog is usually located slightly to the right of the midline, just below the costal margin, it has been our practice to perform the operation on the animal's left side. When the necessity arises, as for instance in cases where a subsequent splenectomy must be performed, the right side is obviously the site of choice. We also make the incision in the abdominal wall as small as possible, contrary to the generally accepted surgical principle of making the incision large enough to permit all necessary manipulations with ease. In our experience the smaller incision has been an advantage, for when the intestine is placed in the body wall incision, there is considerable strain on the sutures closing the remainder of the opening and, as a result, the wound fails to close promptly or may even break open again; use of many stay sutures will not always prevent this.

*First Stage.*—An incision about 4 cm. long is made on the left side at the level of the last nipple lateral to the border of the left rectus muscle and about 5 or 6 cm. below the left costal margin. After cutting the skin, each layer of muscle and fascia is separated by blunt dissection. The peritoneum is incised and the edges are caught with several clamps. The operator's index finger is then inserted into the abdomen and an attempt made to locate the cecum. This frequently proves to be very difficult because of the small opening; often long sections of small intestine must be drawn out before the cecum can be located. As soon as the cecum has been brought into the operative field, it is kept care-

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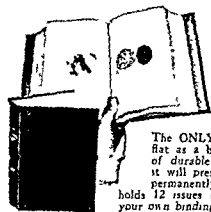
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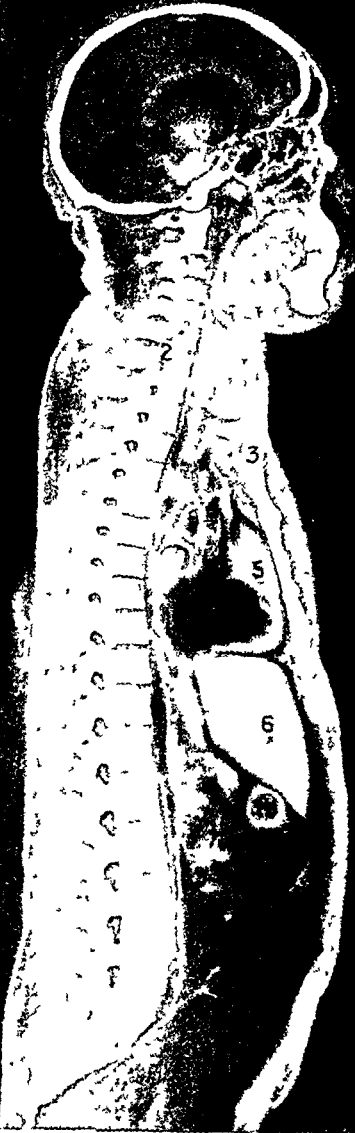
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cecum project beyond the end of the main incision and under the skin for some distance, one insures that the part of the cecum between the final fistulous opening (made in the second stage operation) and the valvular fold is provided with a firm membranous cover, namely, the skin.

The animals are not given any food on the first day after recovery from the anesthesia, and very little water is allowed. On the second day feedings of milk are begun, and on about the seventh day a stock ration. The dressings are changed every other day until the wound is completely healed.

*Second Stage.*—The second stage of the operation is carried out in from three to four weeks after completion of the first. At this time the area of skin where the cecum can be palpated as a long round mass is anesthetized with novocaine solution. At a point nearest the tip and farthest away from the bend in the cecum, a very small puncture wound is made with the tip of a scalpel. A metal cannula is then inserted, and an attempt is made to determine whether the opening is in the cecum. The test of this consists of palpation of the cannula within the intestine. If the test is negative, the wound is enlarged and the test repeated until there can be no question that the cecum has been incised. The edges of the cut cecum usually evert through the wound in the skin and are left in this position. We have observed no tendency for this opening to close, in contrast to our experience with gastric fistulas of the valve type. The animals are allowed to recuperate for about a week before being used for experimental purposes.

#### COMMENTS

We now have 4 dogs with valvular fistulas in the cecum made in this manner. All of our operations were performed over twenty months ago, but the animals are still in good condition. No difficulty has been encountered in maintaining their body weight, nor has there been excess leakage from the cecostomy openings. Two of the dogs show no leakage whatever, while the other 2 pass from 1 to 2 c.c. of intestinal material per day. Erosion of the body wall has not taken place in any of these animals. Experiments involving the insertion of a balloon into the large intestine by way of these fistulas have been carried out without difficulty. Materials of various kinds, including bacterial cultures, have been instilled without any harm to the animals. It is possible to flush out the cecum with any desirable liquid without difficulty. The little finger can be inserted without causing any untoward effects. The presence of the valve can readily be detected by the palpating finger.

#### CONCLUSIONS

A method of preparing a cecostomy with a valve in dogs has been described. Observations over a long period of time show that this type of operation can be carried out very easily and is in every way compatible with maintenance of an excellent state of nutrition and a long normal life in the operated animals.

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transverse bar by a connecting rod (*H*) with a bearing at each end. The excursions of the vertical rods (*A*) are controlled by the length of the windshield wiper arm, which may be made adjustable. Wipers may be obtained for operation on air pressure, vacuum, or by electricity.

Since our present set-up is maintained at body temperature, the board (*B*) is cut to such a length that it rests on shelf supports in a 37.5° C. incubator where it is securely anchored with a screw clamp. The wiper operates on the building air pressure line. The speed of the oscillating paddles is controlled by the quantity of air admitted through a needle valve. The valve is connected with the wiper by means of a rubber tube (*I*) leading into the incubator through a small opening, plugged with a two-holed rubber stopper. The exhaust air escapes from the wiper through tube (*J*) and out of the incubator through the second hole in the rubber stopper. This prevents the escape of cool air in the incubator which would cause fluctuations in the temperature. When the apparatus is in use outside of the incubator, it is clamped between two iron stands.

For mixing the contents of beakers, strips of glass, celluloid, or more recently, flat wooden "tongue-blades" are clamped to the free ends of the vertical oscillating rods (*A*) by the spring type of wooden clothespins. Small flasks may be agitated by fastening them to the oscillating rods by means of rubber bands.

The above described device has many adaptations in the laboratory, is inexpensive, simple of construction, practically noiseless in operation, and permits a reciprocating motion of easily controllable amplitude and rate.

---

## A SECTIONAL TEST TUBE RACK\*

---

FOR USE IN THE TITRATION OF SEROLOGIC AND OTHER BIOLOGIC REACTIONS

---

S. S. LIGHTMAN, M.D., NEW YORK, N. Y.

---

THE sectional test tube rack was devised primarily for convenience in reading hemolytic reactions in connection with bile salt determinations in body fluids by the author's procedure depending on bile salt hemolysis.<sup>1</sup> This type of rack permits direct visualization of each entire row of tubes. Individual tubes do not have to be removed for inspection. In timed reactions this proves advantageous. The apparatus may also be used for the performance of agglutination, precipitation, hemolysis, and the Wassermann reactions.

### DESCRIPTION OF APPARATUS

The rack consists of a stand and individual sections. The dimensions of the stand, length and number of sections, and the number and size of the holes are determined by the use for which the apparatus is intended.

---

\*From the Laboratories and the Medical Divisions of the Mount Sinai Hospital.  
Received for publication, February 21, 1936.

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# IMPROVEMENTS IN THE VARNEY ANAEROBIC JAR\*

GEORGE H. CHAPMAN, NEW YORK, N. Y.

THE Varney anaerobic jar<sup>1</sup> has been in daily use in this laboratory since 1927. In 1929, due to an unusual combination of circumstances, the author received severe burns in handling the phosphorus. However, the unique features of the jar prompted its continued use. Precautions were adopted to prevent further accidents. From time to time, modifications were adopted which resulted in distinct improvements. The present type of apparatus and the method of handling it will be described in this paper.

## DESCRIPTION OF THE APPARATUS

*The Glass Museum Jar.*—The outer glass jar is a cylindrical museum jar similar to that originally used by Varney except that, because of the number of plates cultivated, a 5 by 18 inch jar is preferred. Such a jar will accommodate from 10 to 12 plates. These jars tend to crack near the flange, but this can be minimized by wrapping adhesive tape around the jar immediately beneath the flange. The assembled apparatus should never be carried by means of the metal clamp, as this imposes considerable strain on the flange. Water is poured in the jar to a depth of about one-half inch for absorption of phosphorus pentoxide fumes.

*The Metal Rack.*—The frame is made of Monel metal and heavily japanned. This minimizes corrosion inherent in earlier models. It fits inside the glass jar with sufficient play to allow for variations in the size of different jars. One of the upright strips of the frame is hinged at the lower end to permit filling the rack with Petri dishes. The feet extend  $1\frac{1}{2}$  inches below the base to keep the dishes out of the water. The top shelf of the rack is fastened to the frame and should be covered with bibulous paper. Metal straps extend  $2\frac{1}{4}$  inches above the top and are used for lifting the rack in and out of the glass jar.

*The Phosphorus Cup.*—The space above the top platform of the metal rack accommodates the phosphorus container. By using a tall container for the phosphorus, reaching within one-half inch of the lid of the museum jar, there is little danger of the ignited phosphorus escaping into the jar. To keep the phosphorus as dry as possible, porous porcelain is used. Battery cups measuring 75 by 38 mm. sold by the Arthur H. Thomas Company as porous porcelain battery cups (No. 4378) have proved to be quite suitable. This cup can be manipulated with much less danger than the original Varney phosphorus assembly.

\*From the Clinical Research Laboratory, 604 Fifth Avenue.  
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fully bathed in warm physiologic saline solution. If this is not done and the organ is allowed to become cool, it rapidly contracts to about one fourth of its normal size and presents great difficulty in the suturing process during the later stages of the operation.

The cecum is then dissected away with scissors from its mesenteric attachments to the small intestine. When the cecum has been completely separated from all of its attachments, it is placed in the incision and allowed to assume as nearly natural a position as possible. This position is not always the same. Sometimes the tip of the cecum shows a tendency to point cephalad and it other times caudad. The direction in which it points aids materially in deciding the best position in which to place the cecum in the incision for suturing.

If it points cephalad, then the suturing should begin with the cecum placed in the caudal end of the incision, if the more natural position makes the organ point caudad, it should be sutured into the cephalad end of the wound. The advantage of this will be apparent when the sutures and procedures described below are considered. This point will be referred to later.

The cecum is drawn into the operative wound until it projects about 4 cm through the body wall. Four or 5 sutures of No. 00 chromic catgut are taken in the base of the cecum on the medial side. These sutures penetrate the peritoneum, muscle and fascial sheaths of the body wall and the muscle layer of the cecum. When these sutures have been placed, the cecum is grasped by its tip and rotated so as to point in the opposite direction. This procedure produces not only a twist in the cecum but also a fold where the organ is bent over on itself, the production of the valve depends upon the successful maintenance of this twist and fold by suitable sutures. The cecum is held in position by the assistant while the operator places 4 or 5 sutures through the peritoneum, muscle and fascia of the body wall and the muscular layers on the lateral side of the cecum. Sutures are then placed along the base of the cecum and wherever may seem necessary until the peritoneal incision is entirely closed, and the part of the original incision not occupied by the cecum is properly closed. The skin is separated from underlying fascia for some distance at the point where the tip of the cecum lies, a suture of No. 00 chromic is then passed through the tip and then fastened to the abdominal muscle and fascia in the tunnel under the skin at the greatest distance possible without unduly stretching the cecum or injuring it in any way. This suture is designed to anchor the tip of the cecum in the cavity under the skin. The remaining edges of the skin incision are then fastened to the top of the cecum with No. 00 chromic catgut, care being taken to see that no sutures are so placed as to be subject to strain which will result in tearing the cecal issue. This terminates the first stage of the operation.

In a preceding paragraph reference was made to the advantage of placing the cecum in either the caudal or cephalad end of the body wall incision, depending upon what seems to be the natural position for the organ to assume. It should now be evident that, after having first anchored the cecum by stitches placed medially, then rotating the organ and maintaining it in that position by sutures properly placed, a portion of the cecum will extend beyond the end of the main incision or not, depending on whether the base of the cecum is sutured to tissues at that end or not. By so arranging matters as to make the exposed

the jar to the incubator by putting one hand under the bottom of the jar while steadying it with the other hand.

*Unloading the Jar.*—Have ready a 500 c.c. Griffin form beaker containing about 200 c.c. of water. Loosen the metal clamp from the jar and carefully pry off the glass lid. Immediately pour water into the porous cup. This prevents further ignition. This must be done quickly or the phosphorus will burn violently. Remove the battery cup by means of crucible tongs and immerse it in water. Very little residue is left in the cup after ignition because most of the combustion products are water soluble. The residual phosphorus in the bottom of the cup is left immersed in water ready for the next test. The porous cup is always kept under water until ready for use when it is drained off. The moist phosphorus is still capable of being ignited immediately by means of magnesium ribbon.

Several of these jars have been in daily use and have given complete satisfaction. They are admirable for anaerobic plating methods.

#### REFERENCE

1. Varney, P. L.: A Simple Method for Cultivating Anaerobes by Means of Phosphorus, *J. LAB. & CLIN. MED.* 11: 1183, 1926.

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## A SIMPLE AND RAPID METHOD FOR OPENING SYRINGES CLOTTED WITH BLOOD\*

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**S**YRINGES in which blood is allowed to clot often must be discarded because of the inability to remove the plunger. The following simple technic, which is an adoption of an old household principle, has been found very valuable in opening and cleaning "stuck" syringes.

Two pieces of soft rubber tubing, each 30 to 40 cm. in length (such as are used for tourniquets), are the only equipment required. The first tube is wrapped tightly around the barrel of the syringe in a counterclockwise direction so as to cover the whole barrel with one or more spirals of flattened tubing. This is then held tightly with the left hand while the second tube is wrapped tightly around the handle of the plunger in a clockwise direction, piling up to several thicknesses which can readily be grasped in the right hand. Force is then applied with the two hands to produce a clockwise rotation of the plunger within the barrel. Usually very little force is required but, if necessary, a powerful, steady torsion can be applied because of the improved grip and increased leverage. Careful and complete winding of both tubes will prevent injury to the operator if a breaking of the glass should occur.

This method has been employed successfully in opening syringes of all sizes in which dried blood has been allowed to stand even for many days.

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\*From the Medical Service, Presbyterian Hospital.  
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## A SIMPLE AGITATING DEVICE\*

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A DEVICE capable of simultaneously stirring the contents of a number of beakers, or agitating small flasks may be readily and simply constructed from wood or metal strips, using an ordinary automobile windshield wiper as the source of power.

We have had such a device in use for over a year constructed of thin oak slats 1 cm. wide, with accommodations for four flasks or beakers. The size of

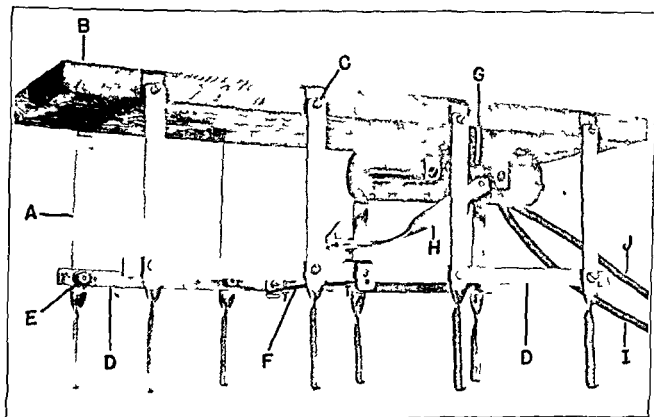


Fig. 1—Photograph of an agitating device operated by a windshield wiper

the apparatus may be varied to suit the purpose at hand. The one that we are now using is primarily for the purpose of stirring the contents of eight 600 ml. beakers simultaneously. It is shown in Fig. 1. Eight brass strips (A) 20 cm. long by 12 cm. wide, twisted at right angles in the lower third, are suspended from a rectangular board (B) by means of suitable bearings (C) so as to be free to move from side to side. The strips are spaced 10 cm. apart. A brass connecting rod (D) joins each set of four vertical strips with freely working bearings (E). The two connecting rods are joined by a rigid transverse bar (F). The reciprocating action of a windshield wiper arm (G) is communicated to the

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able to dispense with all dressings and alkali by the use of a permanently placed rubber catheter. The type used is either a wing or mushroom self-retaining catheter. It is placed in the pouch by means of a stylet. Fig. 1 illustrates the catheter in position in the pouch. The tube is first introduced after the dog has completely recovered from the effects of the operation. It is important that the tubes fit the pouch openings very snugly. For the larger openings a number 34 or 36 catheter has been found very satisfactory. About two inches of the tube stem is allowed to project beyond the body-wall. If the tube tends to be drawn into the pouch, one can prevent this by the use of a safety pin through the tube stem, or by placing a skin suture through the tube. Most animals ignore the presence of the tube, but an occasional one will develop the habit of pulling it out. This can quickly be discouraged by a skin suture which will keep the tube in place for days to weeks.



Fig. 2.—Photograph of gastric pouch opening with tube in place

A very minimal amount of circumscribed erosion of the tissue immediately around the tube may occur due to peritubular leakage of secretion. This remains quite superficial and rarely extends more than a few millimeters beyond the mucosal junction. It does not progress and needs no special care. Occasionally a mild skin burn may occur on the hind legs due to the posture assumed in recumbency. The animals soon learn to avoid such occurrences.

An added convenience is the fact that this self-retaining catheter can be used for collection of samples. It is likely that the constant presence of these catheters may slightly increase the combined acid values. In two sacrificed dogs that had such catheters in situ for one and two months, respectively, an examination of the pouches revealed absolutely no noticeable mucosal changes.

To date we have employed the above device in five Pavlov and Heidenhain pouch dogs with complete success. It has also been used satisfactorily in an

*The Stand.*—(Fig. 1.) The stand consists of a base and uprights and is made of noncorrosive metal if immersion in water is intended. The upper edge of the uprights permits suspension of the trays and shaking of their contents without dislodgement.

*The Sections.*—Each section is as wide as the diameter of the test tubes to be used. This permits close apposition of an entire row of tubes to the frosted glass surface of an illuminating box for precise differentiation between partial and complete hemolysis if an artificial source of light is used. Holes to accommodate agglutination or hemolysis tubes are punched in metal strips as close as possible to each other to facilitate comparison of the contents of adjacent tubes. The margins of the metal strips, bent at right angles tangentially to the

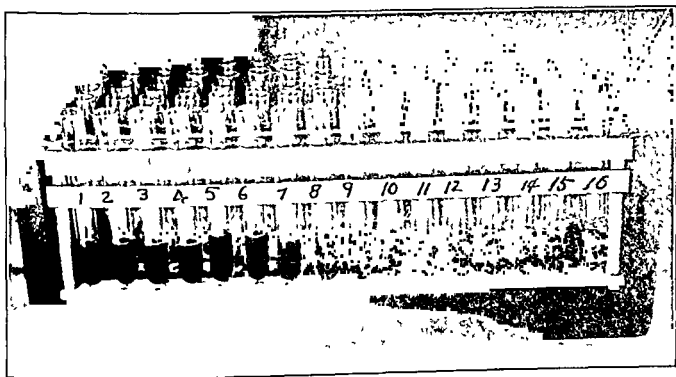


Fig. 1.—Rack with five sections.

holes, serve as flanges. A similar strip of metal with identical punching of holes is juxtaposed, their flanges almost meeting. A free view of the lower half of the tubes is thus afforded and the tubes are secure from falling out when their contents are agitated. The tubes rest on a platform. In agglutination or other procedures where the character of the sediment is examined through the bottom of the tube, holes may also be punched in the platform. Each section rests suspended on the uprights of the stand. The upper metal strip projects beyond the uprights of the stand to provide means for raising each section off the stand. Noncorrosive materials are used throughout.

#### REFERENCE

1. Lichtman, S. S.: A New Procedure for the Estimation of Bile Salts in Body Fluids Based on Bile Salt Hemolysis, *J. Biol. Chem.* 107: 717, 1934.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## THYROID FUNCTION, Iodine Tolerance Test for the Investigation of, Watson, E. M., and Barber, A. S. *Endocrinology* 20: 358, 1936.

An iodine tolerance test is described whereby the relative rate of disappearance from the blood stream of intravenously injected iodine may be expressed. The results of the application of the test in 30 cases are recorded.

In 13 of 16 individuals with clinically normal thyroid glands, from 9 to 23 per cent of the injected iodine remained in the blood stream 6 hours after its injection. In 6 of 10 patients with thyrotoxicosis not any of the injected iodine remained in the blood stream after six hours and in none of these cases was more than 5 per cent of the injected iodine found to be present at this time. In 4 cases of hypothyroidism the average quantity of iodine in the blood 6 hours after its injection was greater than normal.

These few observations suggest that the iodine tolerance test may be a means of providing evidence of diagnostic importance in cases of doubtful thyroid disease.

The technic employed for performing the iodine tolerance test is briefly as follows. With the patient in the fasting state in the morning, an amount of Lugol's solution containing 250 $\gamma$  of iodine per kilogram of body weight, after being diluted with 15 c.c. of 0.85 per cent NaCl solution, is injected intravenously. Samples of venous blood of about 12 c.c. each are obtained immediately before the injection and five minutes, two, four, and six hours afterward. These samples are received in tubes containing a small amount of potassium oxalate which serves as an anticoagulant. Food is withheld from the patient during the test period.

The concentration of iodine in each sample of whole blood is estimated by means of a method described by Perkin. In this procedure 10 c.c. of blood are placed in a nickle crucible together with 2 gm. of potassium carbonate and combusted on a hot plate and in a muffle furnace for four and one-half hours. The charred mass is extracted with alcohol, filtered and the filtrate is evaporated to dryness. The residue which remains is dissolved in water and when the solution is made slightly acid with  $H_2SO_4$  and a drop of freshly prepared bromine solution is added, the iodine is oxidized to iodate. The addition of potassium iodide frees the iodine which is estimated by titration with 0.001 N sodium thio-sulphate solution with starch serving as an indicator.

The iodine content of the blood specimen secured five minutes after the injection of the Lugol's solution minus that of the preliminary control sample, is regarded as representing the maximum increment caused by the injected iodine and is consequently recorded as 100 per cent. With this value as a basis, the findings for the other samples are expressed accordingly. While the results so obtained represent the relative rather than the absolute iodine concentrations, they do provide an indication of the rate of disappearance from the circulating blood of the injected iodine in a specified time.

## KIDNEY, Hypernephroid Carcinoma of, With Tumor-like Thrombus Filling the Inferior Vena Cava and Right Heart Cavities, Woodruff, L. W., and Levine, V. J. A. M. A. 106: 1544, 1936.

A case of tumor-thrombus of the inferior vena cava, arising from a hypernephroid carcinoma of the kidney and extending into the right atrium and ventricle, was not diagnosed during life, largely because of the absence of edema of the lower extremities. Circulation time tests might make a correct diagnosis possible in this type of case.

METHOD OF IGNITING THE PHOSPHORUS

The most convenient form of phosphorus is that sold by the J T Baker Chemical Company under the label "Phosphorus, U S P, yellow, sticks, 3/16 inch" which is cut into convenient lengths. One of these pieces (about 1 inch long) is sufficient for a 5 by 18 inch jar.

The phosphorus is ignited by means of magnesium ribbon cut about 1½ inches long. It is best to wear "sun glasses." Ignite the ribbon in a Bunsen flame and put the incandescent magnesium into the cup containing phosphorus. The phosphorus will ignite immediately. Because of the violent reaction which follows, the lid of the jar must be put on immediately.

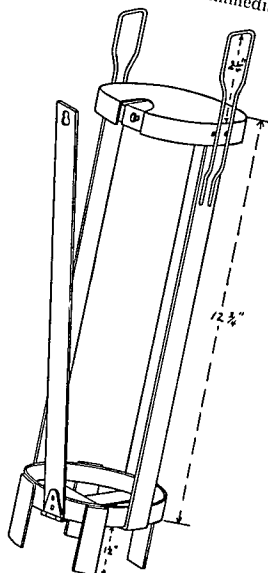


FIG 1

## ASSEMBLING THE JAR

*Loading*—The Petri dishes containing the cultures are interleaved with bibulous paper. They are stacked inside the metal rack which is then closed and lowered into the glass jar. Place the porous cup on the top shelf and, by means of long forceps, place a stick of phosphorus inside the porous cup. The phosphorus will not ignite for a while. Adjust the rubber gasket. Have the lid ready to set in place without delay. Ignite the magnesium ribbon and drop it into the porous cup. This ignites the phosphorus almost immediately. Cover quickly with the lid and clamp it tightly. Avoid drafts of cold air. Transport

In rabbits lightly anesthetized with ether, a short incision is made in the scalp and a tiny opening is drilled in the skull at a point 2 mm. lateral to the sagittal suture and 1.5 mm. anterior to the lambdoidal suture. Through this opening, 0.4 c.c. of sterile suspension is slowly injected into the occipital lobe to a depth of about 3 mm. by means of a short fine needle. The skin is then sutured and the wound covered with collodion. In addition, 0.6 c.c. of the inoculum is injected into the marginal ear vein. The rabbits are weighed before the inoculation and daily thereafter.

*Positive Test.*—After an incubation period of from two to six days (in one rabbit, sixteen days), slight impairment of locomotion sets in, followed by a progressive spastic paralysis of the hind limbs. The gait becomes decidedly ataxic, and the animal may stagger and fall. In the more marked reactions, retraction of the head, opisthotonos and convulsive movements are observed. Van Rooyen reported nystagnus, grinding of the teeth and urinary and fecal incontinence in some animals. Progressive wasting and weakness, affecting particularly the hind quarters, are found in all cases. Death occurs frequently within three days to one month, usually in ten days. A larger number recover completely or pass into a chronic state.

At autopsy, gross examination reveals only a slight hyperemia of the meninges. Microscopically, usually little or no cellular infiltration in the brain is found, but with more active material Gordon noted a lymphocytic meningitis and perivascular lymphocystic infiltration of the brain substance. No inclusion bodies have been described. Cultures of the brain and meninges are sterile.

**DIARRHOEA, Infectious, An Epidemic of, in the New-Born, Barenberg, L. H., Levy, W., and Grand, M. J. H. J. A. M. A. 106: 1256, 1936.**

An epidemic of infectious diarrhea, parenteral in origin, occurred, thirty-two infants presenting a characteristic syndrome.

There was no relationship between the normal type of feeding and the incidence of the disease.

Dehydration was evident, but true alimentary intoxication was noted in only one instance.

Otitis media and bronchopneumonia as complications were frequent.

The severity of the disease decreased with the return of alkaline stools.

Bacteriologic studies failed to reveal a causative organism.

The administration of 1 per cent salt solution orally was well tolerated and obviated the use of the intravenous route for nutrition or medication.

Necropsies did not show a pathogenic picture in the gastrointestinal tract to indicate a primary intestinal infection.

The finding of pneumonia with mononuclear infiltration in the absence of any intestinal lesions suggested the possibility of a virus infection.

**RENAL CARCINOMA, Prognosis in, Braasch, W. F., and Griffin, M. J. A. M. A. 106: 1343, 1936.**

As an index to prognosis and in the interest of simplified terminology it would seem best to regard all malignant tumors of the renal cortex as carcinomas and to grade them according to the degree of cellular differentiation.

From a clinical point of view, carcinoma of the renal cortex may be divided further into two groups which have distinct clinical characteristics; namely, adenocarcinoma (hypermephroma) and alveolar carcinoma.

Although they may be various factors, such as metastasis, which can modify post-operative results, the prognosis usually will conform to the histologic evidence of malignancy.

Metastasis occurs most frequently with renal tumors of the higher grades of malignancy and may be present without causing clinical evidence. Lymphatic extension is a frequent occurrence. Metastasis is found most often in the lungs, rather infrequently in the osseous system. Although the progress of the disease in the presence of pulmonary

# A SIMPLE CATHETER DEVICE FOR THE CARE OF GASTRIC POUCH ANIMALS\*

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EVERY investigator who has prepared gastric pouch dogs has been faced with the problem of controlling the erosion of tissue around the pouch opening caused by the digestive activity of the gastric juice. One of the usual methods employed in the postoperative care of such animals is the use of alkali to neutralize the acid of the pouch secretion, thus preventing peptic

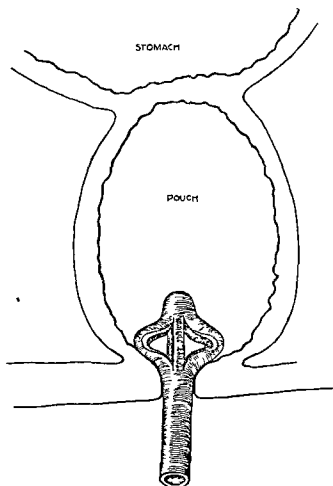


Fig. 1—Schematic drawing of a self-retaining wing catheter in a gastric pouch

digestion. Magnesium oxide, for example, is placed in gauze packs and kept in position over the fistula opening by means of abdominal binders of various types. Daily dressing is needed as a rule, and when the pouch is large or when the animal is fed a diet which stimulates excessive secretion, it is often necessary to change dressings twice a day in order adequately to control erosion. Despite the best of care, ulcerations do occur, and if the animal is by chance neglected for a few days, erosion through to the peritoneum is not unlikely.

It is obvious that such care is burdensome and time consuming, especially so when one has several pouch animals requiring attention. We have been

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tacosis. Only very rarely are necrotic patches on the liver to be seen. The spleen is enlarged to a variable extent. Smears should be made from the inflammatory exudate and stained by Castaneda's method. The stain is prepared as follows:

Phosphate buffer solution (pH - 7.0)	95 c. cm.
Formalin	5 c. cm.
Löffler's methylene blue	10 c. cm.

Smears are stained with this for two minutes, without preliminary fixation, washed with distilled water, and then counterstained with 1 per cent aqueous safranin. Examined under the oil immersion lens, the specific psittacosis bodies (L.C.L. bodies) appear as blue granules, about 3 microns in diameter, mostly in small clumps within mononuclear cells. In a properly stained smear all other components stain pink. Bacteria also stain blue in many instances, but are easily recognized by their size and shape.

If mice do not appear sick by the tenth day, it is advisable to kill and examine them. If nothing abnormal is visible and the spleen is not enlarged, they may be considered uninfected. If the spleen is enlarged and there is a suspicion of peritoneal exudate on some of the peritoneal folds around the lesser omentum, but no L.C.L. bodies in smears, it is advisable to emulsify the spleen and subinoculate it into two fresh mice. Cultural tests of the spleen should be made at the same time. If the virus is present, this second mouse passage will give typical findings.

The virus is readily inactivated, and if material has to be sent some distance to the laboratory it is advisable to pack it with ice, preferably in a thermos flask, during transit.

**LIVER, Clinical Value of Test for Hippuric Acid in Disease of the,** Quick, A. J. Arch. Int. Med. 57: 544, 1936.

The excretion of hippuric acid after the administration of sodium benzoate was studied in a series of one hundred cases, in fifty-eight of which hepatic or biliary disease was present. A patient with a normally functioning liver excretes approximately 3 gm. of benzoic acid in the form of hippuric acid in four hours after taking 6 gm. of sodium benzoate. A low output of hippuric acid occurs in cases of catarrhal jaundice and various forms of hepatitis and usually in cases of a malignant process with metastasis to the liver, syphilitic cirrhosis and atrophic and hypertrophic cirrhosis. Reaction to the test is normal in cases of cholecystitis, cholelithiasis and biliary obstruction due to stones in the common duct if the condition is of short duration. The test appears as a promising means for estimating hepatic insufficiency and as an aid in the differential diagnosis, especially in distinguishing between the jaundice of hepatitis and the jaundice arising from obstruction due to a stone in the common duct. The test is useful in following the course of disease of the liver and in determining the effectiveness of therapy.

**TONSIL, Malignant Lymphoma of,** Jackson, H., Jr., Parker, F., Jr., and Brues, A. M. Am. J. M. Sc. 191: 2, 1936.

In fifteen years there have been admitted to the Huntington Hospital 236 cases of malignant disease of the tonsil. Of these, 28 (11.8 per cent) were proved to be malignant lymphoma of one type or another.

The most prominent symptoms initially noted by the patients were persistent sore throat, swelling within the throat and enlargement of the cervical lymph nodes.

Of these patients 56 per cent developed generalized lymphoma.

The excellence or rapidity of the immediate response to radiation is no index of the ultimate outcome.

The average duration of life from the onset to death was 2.6 years, the median 1.4 years, and the extremes were 3 months and 13 years.

One patient was alive 5 years, 2, 10 years and 1 alive and free from symptoms 18 years after the onset.

antral pouch in a swine. One dog with a pouch secretion rate of 125 cc per hour at the peak in response to its daily meal has been maintained without dressings of any character entirely free of disturbing erosion for a period of over six months. In addition we have been able rapidly to heal extensive and deep ulcerations occurring in animals despite daily dressings with alkali. Fig. 2 illustrates the excellent condition of the tissues surrounding the self-retaining catheter in a Pavlov pouch dog.

The authors are indebted to Dr. Louis N. Climborn of the Department of Surgery for the suggestion that such a device might prove of value.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va

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### **Delafield and Prudden's Textbook of Pathology\***

THIS is a book which needs no introduction, having been for fifty years one of the best known and most popular of all reference texts in pathology.

It is the one text on this subject to which this reviewer has never turned without finding something pertinent to the problem at hand.

The present revision by Dr. Wood is, as he says, in one sense a jubilee publication, the first edition having appeared in 1885.

It will be found thoroughly revised, especially in the section on the Nervous System, and the present volume, as heretofore, remains one of the most useful of all reference texts on this subject.

As always, Delafield and Prudden can be recommended not only without reserve, but with enthusiasm.

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### **The Bacteriology of Typhoid, Salmonella, Dysentery and Carrier States†**

AS DR. HAVENS remarks, in the opening chapter of this book "There is, perhaps, no more ubiquitous group than the *Salmonella* and dysenteries, nor one that offers, on the one hand, such technical pitfalls in its study and, on the other, so much satisfaction when these technical difficulties are crowned by successful isolation and identification."

Few there are who have ever been confronted with these problems who will hesitate to agree with this summary of the difficulties they present, and all within whose province such problems lie will welcome this small but excellent book devoted to methods for their solution.

In view of the unwarranted degree to which clinical reliance has been and still is placed upon agglutination reactions are accepted as diagnostic of these infections, the book begins with a clear description of the antigenic composition of the bacterial cell.

Then follows a discussion of bacterial variation, particularly as related to antigenic variations. There is an excellent discussion of the culture media best suited to the isolation of pathogens from fecal specimens, the procedures cultural and serological applicable to the identification of intestinal pathogens, after which the various organisms are discussed in detail. The carrier problem is discussed at length.

Bearing on every page inherent evidence of the extensive experience of the author, it is certain that this volume will become a most valuable reference text for the laboratory worker, the health department, and the clinical laboratory in general.

It may be recommended without reserve as a valuable and most useful contribution on a subject of general importance.

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\*Delafield and Prudden's Textbook of Pathology. Revised by Francis Carter Wood, Director, Pathological Department of St. Luke's Hospital, New York, etc. Cloth, 1406 pages, 22 plates and 839 text illustrations. William Wood & Co., Baltimore, Md.

†The Bacteriology of Typhoid, Salmonella, Dysentery, and Carrier States. By Leon C. Havens, M.D., Director of Laboratories, Alabama State Department of Health. Cloth, 158 pages, 6 figures. The Commonwealth Fund, New York.

**PNEUMOCOCCIC INFECTIONS** Type Specificity in Pneumonia and Kohl C, and Reitzel E J J A M A 106 1557, 1936

In an interval of three years, from December 1934 to December, 1935, pneumococci were found in 353 patients, of whom 322 adults (91 per cent) had pneumonia.

During the last twenty-three months of this study diagnostic cultures of all available types, that is, I to XXXII, were used. Eighteen types other than the original three were found in either pneumonia or other infections. Only two strains were found that failed to be classified in Types I to XXXII.

In pneumonia, Type I was the most frequently encountered type having been found in 138 (42.8 per cent). The order of prevalence of the other types was: Type II, 12.1 per cent, Type III, 8.5 per cent, Type VII, 1.7 per cent, Type VIII, 2.4 per cent.

The incidence, bacteremic rates and mortality of Type I (12.8, 49.2 and 44.1 per cent respectively) has been found to be higher than elsewhere.

Seventeen types other than the original three were found in fifty cases of pneumonia. From this small series conclusions cannot be drawn as to the individual clinical characteristics of these types in this locality.

The Neufeld method for typing has been found to be satisfactory.

**ALCOHOL ADDICT**, Observations on the Etiologic Relationship of Vitamin B to Polyneuritis in the, Jolliffe N, Colbert C N and Joffe P M Am J M Sc 191 51, 1936

The vitamin B and caloric intake of 42 alcohol addicts, 26 of whom had polyneuritis, have been estimated quantitatively by Cowgill's formula. When the first 1,600 calories contained in the alcohol consumed by each subject are included in the estimation of the vit/cal ratio a definite correlation is found between the inadequacy or adequacy of the vitamin B intake and the presence or absence of polyneuritis. This correlation is as follows:

- 1 Every alcohol addict with polyneuritis had an estimated inadequate vitamin B intake.
- 2 No alcohol addict with an estimated adequate vitamin B intake had polyneuritis.
- 3 Every alcohol addict with estimated absolute deficiency of vitamin B for twenty-one days or more had polyneuritis.
- 4 Polyneuritis may develop in an alcohol addict as early as the 7th day of estimated absolute deficiency of vitamin B.

The authors' data substantiate the hypothesis that polyneuritis in the alcohol addict is due to the lack of vitamin B by demonstrating (1) that the diet consumed by alcohol addicts with polyneuritis failed quantitatively and over a sufficient period of time to contain adequate amounts of vitamin B as compared with the predicted requirement, and (2) that the diet consumed by alcohol addicts without polyneuritis, though the addiction was of long duration, contained quantitatively adequate amounts of vitamin B as compared with the predicted requirement.

It is therefore concluded that alcohol has no direct toxic action (chronic) on the peripheral nerves, and that polyneuritis in the alcohol addict is due to vitamin B deficiency.

**HODGKIN'S DISEASE**, The Gordon Test for, Rosenberg D H and Bloch L J A M A 106 1156, 1936

**Method**—With aseptic precautions a lymph node is collected in a sterile test tube or Petri dish. One or more grams is placed in a small sterile mortar under cover, and the rest of the node is kept in a refrigerator. This tissue is cut into very small pieces and is ground into a fine emulsion nutrient broth of pH 7.1-7.2 being added to make a 1:10 suspension. It is maintained in a refrigerator at from 0 to -4° C for seven to ten days, and, before using, is tested for sterility aerobically and anaerobically. All lymph nodes in this study were obtained from live patients. If necropsy material is studied, the node should be removed aseptically, disinfected with absolute alcohol, and dipped into boiling water and then into ether. Phenol (0.5 per cent) may be added to the broth as a further precaution against contamination.

ment of the underlying condition responsible for its production, Dr. Balme wisely emphasizes that pain is, after all, a symptom and a sign and recalls the dictum of Hilton that "every pain has its distinct and pregnant signification if we will but carefully search for it."

As the author says, further: "The call of pain presents a double appeal to every conscientious physician. It is at once a call to investigation and a call to relief and neither can be truly obeyed without the fullest recognition of, and response to, the other."

The first part of the book discusses the problem of pain, its nature, pathway, its relation to physical phenomena, the sensitivity of the individual to pain, its classification, and methods for the investigation of pain.

Part II discusses general and systemic pain together with methods for its control, while Part III discusses regional pain and methods for its relief.

Part IV is devoted to the therapeutics of analgesia.

The book is well and clearly written and bears the hallmark of evaluated experience and critical analysis of the pertinent literature. There is a comprehensive index.

This book can be recommended as an eminently useful and practical text.

### Parenteral Therapy\*

THIS book is, apparently, an outgrowth of one first written by the senior author in 1924 under the title of "Intravenous Therapy."

As the title indicates, its purpose is to present all methods for the administration of medicaments except the alimentary route.

The first section (155 pages) presents in detail and with excellent and ample illustrations the general technic of parenteral therapy. This section should be of great practical value because of its clear and full descriptions of technic.

The second section is a therapeutic index in which a large number of conditions are listed alphabetically each followed by a list of drugs, etc., applicable to their treatment.

The third section under the heading pharmacologic notes presents an alphabetical list of drugs and various preparations which may be administered parenterally, giving their action, dose, and method of administration.

A list of their distributors is appended.

The book is well indexed and in a relatively small space embodies a large amount of information of practical value. It should be favorably received and should prove of great usefulness to all who may from time to time desire to administer medicaments parenterally and thus to physicians, dentists and veterinarians.

### Erratum

In the article "Schilling's Hemogram" by Emil Maro Schleicher in the September, 1936, issue of the JOURNAL, the last sentence on page 1298 should read: "From the data obtained, the relative average ratio per low power field is determined by adding the columns and dividing the total numbers into each other."

\*Parenteral Therapy. By Walter Forest Dutton, M.D., Polyclinic and Medico-Chirurgical Hospitals, Graduate School of Medicine, University of Pennsylvania, etc., and George Burt Lake, M.D., Formerly Special Lecturer in Hygiene, Purdue University, etc. Cloth, 386 pages, 90 half-tones and engravings. Formerly Medical Director, Charles C. Thomas, Springfield, Ill.

metastasis may be delayed by nephrectomy and irradiation of the lungs postoperative results indicate that the chance for recovery is so slight that operation is not justified. It is possible that the roentgenographic evidence of pulmonary metastasis in some of the few cases in which patients were reported as having recovered was incorrectly interpreted.

Calcification of tissues in cases of renal adenocarcinoma is a frequent occurrence and indicates a favorable prognosis. Urographic evidence of widespread involvement of all calices and of the pelvis indicates a high grade of malignancy and a guarded prognosis. The excretory urogram will give accurate information with regard to the presence of renal neoplasm in a high percentage of cases. Its routine use should be more frequent in the identification of abdominal tumors.

Although clinical data suggest that suprarenal elements may be included in some hypernephromas to account for the vascular manifestations such as hypertension and telangiectasis, neither chemical analysis of the tumors nor postoperative clinical data corroborate such an assumption.

While the size of the tumor alone has no bearing on the late postoperative results, nevertheless a large, fixed tumor together with a short history and evidence of marked toxemia would indicate a bad prognosis and would accordingly render the advisability of operation questionable.

#### TRICHINOSIS Laboratory Diagnosis in Heathman L S Am J Hyg 23 397 1936

In the author's hands the intradermal skin test and the precipitin test appear to be of much less value in the laboratory diagnosis than do the eosinophile count together with muscle biopsy, and the study of the meat suspected of being the source of infection.

Intradermal skin tests and precipitin tests in animals heavily infected with *Trichinella larvae* give even a lower percentage of positive reactions than that found in human beings. Animals did not tend to develop positive intradermal reactions regularly after being tested a number of times. The intradermal tests in both man and animals are less clear cut and more difficult to read than a number of other diagnostic intradermal tests.

It would seem very important both from the theoretical and practical standpoint that skin tests in trichinosis be more thoroughly studied.

#### PSITTACOSIS, Laboratory Investigation of, Burnet, F M Med J Australia 1 363, 1936

If the suspected bird is available it should be handled with great care to avoid inhalation of dust from the feathers and feces, and preferably drowned immediately in a solution of some weak antiseptic. The body cavity is opened with aseptic precautions and note made of any lesions present. In acute psittacosis of cockatoos, subacute pericarditis and inflammation of air sac linings are almost invariable. From the semipurulent lymph, smears should be made and stained by Castaneda's method. The liver is usually enlarged and flabby, with usually some variation from the normal color; it may be brownish green or, more rarely, may have yellowish necrotic patches. The spleen is usually moderately enlarged, and should be removed for inoculation into mice. For this purpose it is ground with a little sterile quartz powder or sand and two cubic centimeters of broth or saline solution. After the abrasive has been allowed to settle, quantities of 0.5 c.c. are inoculated intraperitoneally into mice.

The mice should be kept under conditions which will minimize the possibility of infecting the workers in the laboratory. Individual glass jam jars with perforated screw tops for each mouse are satisfactory. If pneumococci are present in the sputum, the mouse will die within three days and may be discarded. If it survives any bacterial infection, psittacosis may be manifested by signs of sickness in the mouse from four days onward. It should be killed as soon as it is obviously sick, and the abdominal cavity examined. Subacute peritonitic lesions with fibrinopurulent accumulations in the phrenic regions and around the spleen are characteristic of Australian strain.

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The absence of local lymph nodes at the time of treatment is in general, of good prognostic import

Lymphoma of the tonsil should be regarded as but one type of malignant lymphoma and the ultimate widespread involvement seen in many patients must constantly be borne in mind

It is suggested that those patients be treated as if they had carcinoma of the tonsil, that is, with very heavy initial radiation with comparable doses for recurrences

**MUCOSA, Are There Cyclic Changes in the Human Vaginal? Zondek B, and Friedmann, M J A M A 106 1051, 1936**

In the human vaginal mucosa the authors could not find cyclic changes analogous to those of the uterine mucosa

The vaginal mucosa shows different microscopic pictures in different places

In deficient ovarian function (primary amenorrhea) is found a picture of a mucosa similar to one with good ovarian function, with even the same changes as in the premenstrual phase

In the absence of ovarian function, by means of ovarian hormones (estrogenic substance, progestin) the authors could produce enlargement of the uterus, a proliferative and premenstrual uterine mucosa and menstruation, but could not find analogous changes in the vaginal mucosa

In human beings the infantile vagina may be influenced by estrogenic substances but it is not certain whether it is because of a specific hormone effect on the vaginal mucosa or on the mucous membranes in general

Since the vagina is developed embryologically different in different species the different reaction of the vaginal mucosa is explainable

**RETICULOCYTES, The Resistance of, to Hypotonic Solutions of Sodium Chloride and of Plasma, Daland, G A, and Zetzel, L Am J M Sc 191 467, 1936**

The absolute number of reticulocytes observed in 100 cases of pernicious anemia, hypochromic anemia, and hereditary or acquired hemolytic jaundice showed no consistent effect in altering the minimum resistance or maximum resistance of the total red blood cell population to hypotonic sodium chloride solutions

Actual enumeration of the relative numbers of reticulocytes and red cells surviving in progressive series of hypotonic solutions of sodium chloride and of plasma showed that, irrespective of the disease present, in some bloods the reticulocytes were more resistant, in some equally resistant, and in some less resistant than the red blood cell population as a whole in the cases studied

Reticulocytes as well as nonreticulocytes vary in age, volume, thickness, hemoglobin content, and probably other physical and chemical properties in the same individual at different times, and in different individuals with varied disease conditions. Thus the fact that young cells are reticulocytes does not necessarily determine their behavior in hypotonic solutions. The reticulocytes may or may not exhibit divergence from the resistance of the cell population as a whole. Such variations may be responsible for the diverse opinions of other observers in regard to the resistance of reticulocytes to hypotonic solutions of sodium chloride and plasma

# Venereal Disease Information

A monthly publication prepared by the U. S. Public Health Service for distribution among the medical profession throughout the United States. It measures approximately 6 by 9 inches and ranges in size from 25 to 75 pages.

It is the purpose of the Public Health Service in issuing this publication to provide in condensed form a monthly summary of the scientific developments in the diagnosis, treatment, and control of syphilis and gonorrhea. More than three hundred American and foreign journals are reviewed for this work. Abstracts are made of articles describing laboratory, pathologic, and clinical work in the field of venereal diseases.

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**Lehrbuch des Stoffwechsels und der Stoffwechselkrankheiten.** Von Dr. med. et phil. S. J. Thannhauser, o. o. Professor der Medizin, Direktor der Medizinischen Klinik der Medizinischen Akademie Dusseldorf. Mit 94 teils farbigen Abbildungen im Text. XX, 741 Seiten. 1929. RM 51.12; gebunden RM 53.82

This volume is a notable work in that it succeeds, to an extent rarely attained, in presenting a very detailed and authoritative account of the known facts of metabolism, without becoming a mere collection of abstracts from the literature. The book is in the German tradition in that it contains much controversial matter, that no pains have been spared to obtain the most recent advances in the various sections, and that its outlook is both scientific and philosophical. Professor Thannhauser has the great advantage of combining a very complete knowledge of the natural sciences with a fine and experienced clinical sense. This combination is not common, and we greet the present volume as a demonstration of the immense advantages gained by the clinician who possesses a thorough scientific training. The treatment of the various subjects is well thought out, and shows the hall marks of a teacher. . . . Many and excellent photographs of cases and diagrams of results abound, and the bibliography makes the volume of incalculable value to the researcher. A translation into English would be of great service to a wider public.

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## Blood Groups and Blood Transfusions\*

AS DR WIENER indicates in his preface, studies of the individual differences in human blood have been extensive and somewhat involved

The practical application of the data evolved not only to the transfusion of blood but, more recently, to various medicolegal aspects such as the identification of blood stains and the determination of paternity, have made it incumbent upon physician and laboratory worker alike to become familiar with the subject. To these this book will come as a welcome contribution.

The reputation and experience of the author in this field assures the reader of a thorough, balanced, and authoritative discussion, an expectation which will not be disappointed.

The subject matter is so arranged and discussed as to furnish all the particular information desired. The advanced worker in this field as well as those to whom it is unknown alike may read this book with profit.

This volume will deservedly take rank as a reference text par excellence in this field.

## Stone in the Urinary Tract†

DESPITE the frequency and importance of calculus formation in the urinary tract, this subject is seldom adequately dealt with in the average textbook.

As a subject of practically monographic proportions it is, hence, best and most adequately discussed in a monograph devoted to it alone. Such an endeavor the author has carried out with no small measure of success.

This book is well conceived and, by virtue of the author's wide and comprehensive experience, well carried out. Beginning with the etiology, chemical composition, and pathology of calculus formation, the text considers in the order named the varied features of renal, vesical, prostatic and preputial calculi with particular attention in each instance to the clinical symptomatology, diagnosis and treatment. A separate chapter is devoted to urinary calculus in children and another to urinary calculus in relation to pregnancy.

The book is well and clearly written and by the use of heavy type, significant portions of the text are brought into relief. The text is copiously and well illustrated. An extensive bibliography is attached covering all the references to urinary calculi from January, 1910 to June, 1928.

This volume should prove valuable to the urologist, the pathologist, and the physician at large as presenting in a clear and comprehensive manner the present information available on this subject. It can be thoroughly recommended.

## The Relief of Pain‡

THIS is a book which should prove of inestimable value to the practitioner. For not only is pain the dominant symptom which drives the vast majority of patients to the physician but it is without doubt the one symptom for the relief of which they are most unanimously clamorous.

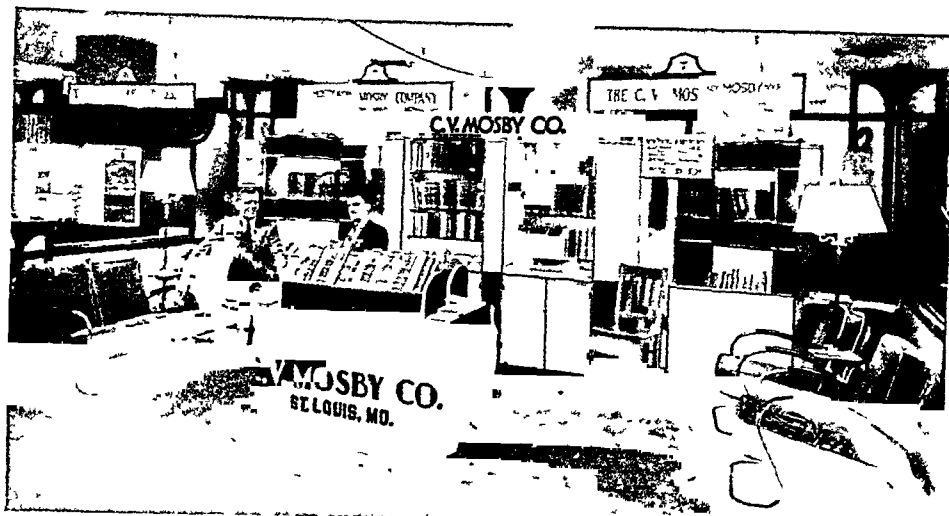
The hints, suggestions, and methods presented in this book should prove, therefore, most welcome to the physician.

It must not be inferred that Dr. Balme discusses pain solely as a symptom to be treated. On the contrary, while its relief is often indicated and of definite importance in the manage-

\*Blood Groups and Blood Transfusions. By A. S. Wiener, M.D. Cloth 220 pp. 41 figures and 71 tables. Charles C. Thomas, Springfield, Ill.

†Stone in the Urinary Tract. By H. P. Winsbury White, Assistant Urological Surgeon, St. Paul's Hospital for Genito-Urinary and Skin Diseases, etc. Cloth 344 pages, 2 colored plates and 181 text figures. P. Blakiston's Son & Co. Inc. Philadelphia, Pa.

‡The Relief of Pain. A Handbook of Modern Analgesia. By Harold Balme, M.D. Formerly Professor of Surgery and Dean of the School of Medicine, Cheefoo University, China. With an Introduction by Sir T. Farquhar Buzzard, Regius Professor of Medicine, University of Oxford. Cloth 392 pages. P. Blakiston's Son & Co. Inc. Philadelphia, Pa.



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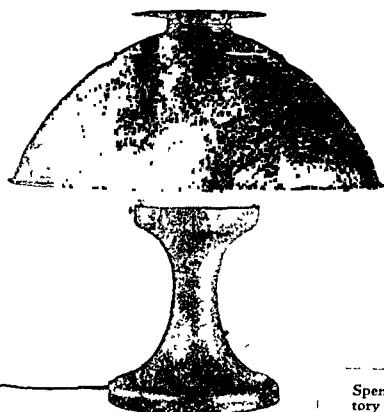
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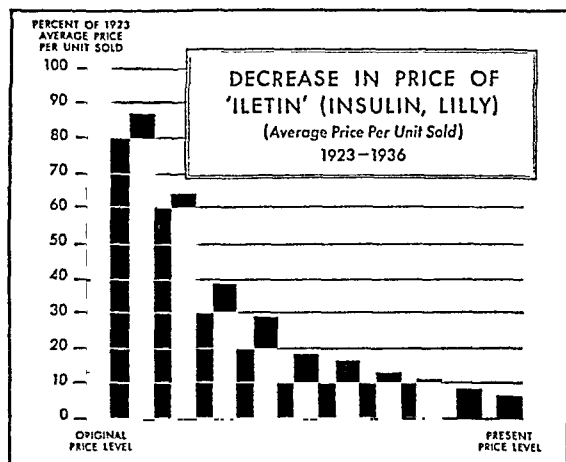
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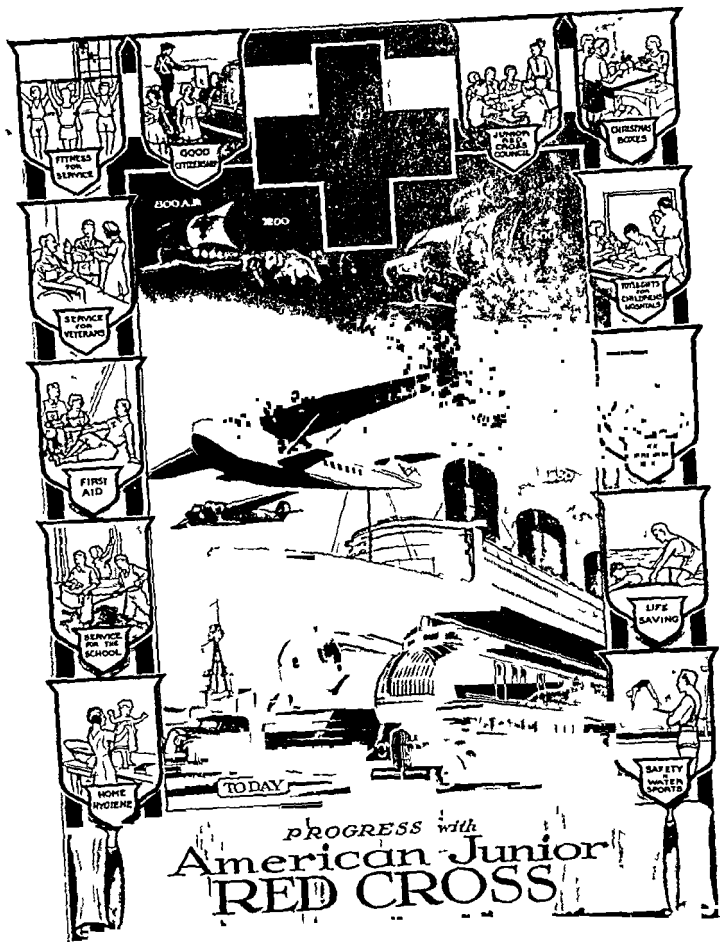
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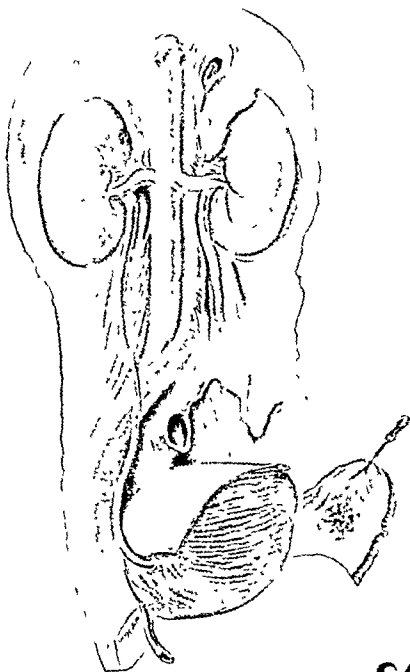
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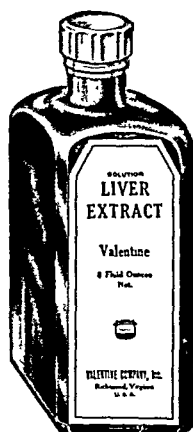
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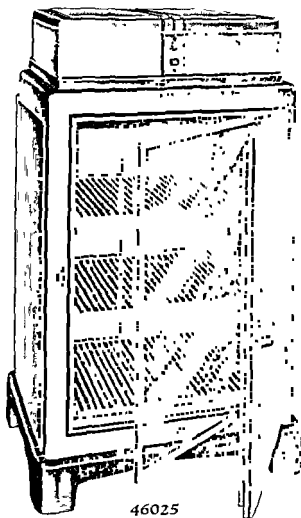
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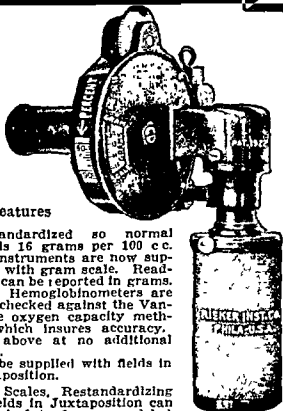
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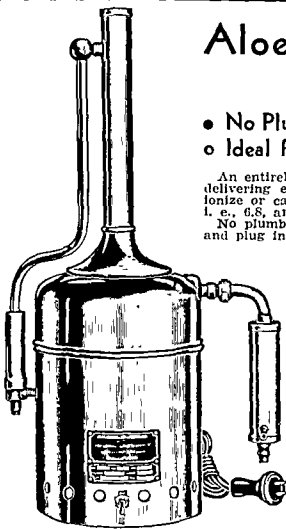
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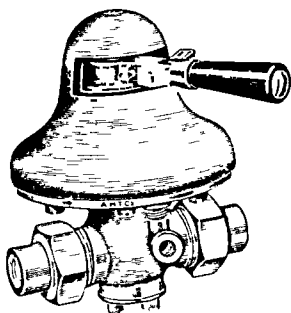
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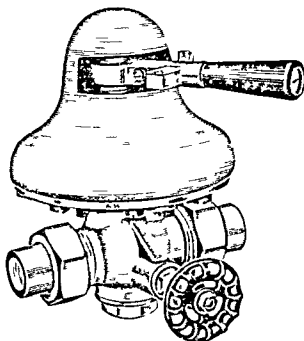
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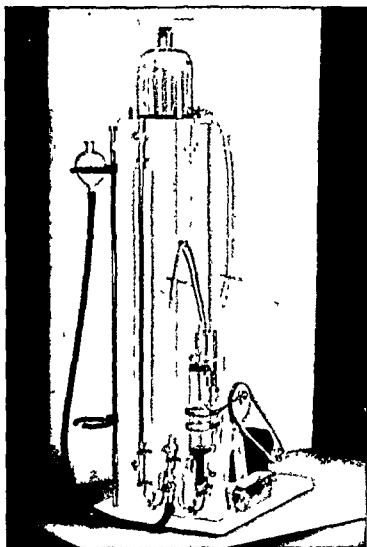
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experiment which utilized the four comparable bone fragments. Each fragment was immersed in sterile fluid of known volume and controlled pH for sixteen hours at a constant temperature of 38° C.<sup>7</sup>

One fragment, the negative control, was immersed in sterile distilled water. A second, the positive control, was immersed for sixteen hours in a calcifying medium of the following composition<sup>5</sup> diluted to give the appropriate percentage of calcium glycerophosphate as noted in the table:

Sodium chloride	0.6	gm. per 100 c.c.
Sodium bicarbonate	0.03	gm. per 100 c.c.
Potassium chloride	0.04	gm. per 100 c.c.
Magnesium sulphate (MgSO <sub>4</sub> ·7H <sub>2</sub> O)	0.025	gm. per 100 c.c.
Calcium chloride	0.010	gm. per 100 c.c.
Tribasic sodium phosphate	0.082	gm. per 100 c.c.
Calcium glycerophosphate	2.0	gm. per 100 c.c.

Enough of the inorganic basal solution was made up at the beginning to serve for all the experiments. The bicarbonate was added last and before this addition the solution was sterilized. Next the pH was adjusted to between 7.4 and 7.6 by equilibration with carbon dioxide.<sup>4</sup> With the addition of glycerophosphate the hitherto inorganic basal solution now becomes the organic basal medium and is thus defined in later paragraphs.

The third and fourth fragments were immersed in the organic basal medium for the same length of time and under identical conditions of temperature and hydrogen-ion concentration but to the medium was added the preparation the effect of which was to be investigated. Allowance was made for dilution so that the salt concentration of the organic basal medium should be identical with that of the positive control.

After the sixteen-hour immersion period the fragments were washed with saline, fixed in 10 per cent formalin, dehydrated, embedded in paraffin and sectioned. The sections were transferred to slides, stained with von Kossa's silver nitrate (1.5 per cent AgNO<sub>3</sub>) for two minutes, then exposed to bright light, and mounted in balsam.<sup>1</sup>

An aqueous extract of the antirachitic principle was prepared by diluting 10 c.c. of viosterol 10,000X, generously furnished by Mead Johnson and Co., with a small quantity of acetone, triturating the mixture with talc, slowly adding water with continued trituration and filtering the product through dry filter paper.

The resultant filtrate was a colorless, slightly opalescent fluid, odorless and tasteless but nevertheless a reliable source of vitamin D as shown by the biological assay already reported.<sup>9</sup>

This aqueous preparation of activated ergosterol which we term aquasterol was added, in these experiments, to the organic basal solution described above. Aqueous control preparations of olive and cod liver oils were made by a technique identical with that used in preparing aquasterol.

With exceptions noted in the table we utilized, for the immersion of bone fragments, a total fluid volume of 5 c.c., representing 0.05 gm. or such amount of

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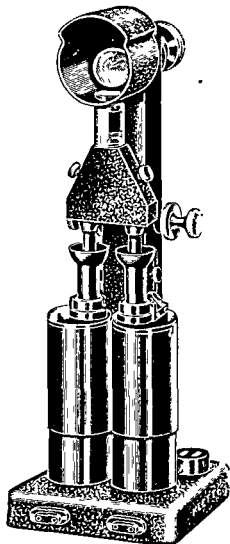
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end of a long bone presents, on the shaft side of the diaphyso-epiphysial plane or zone 1, reserve cells of Dodds and Cameron,<sup>2</sup> a zone of proliferating cartilage formed by parallel columns of cells (zone 2, multiplying cells, Dodds and Cameron). Proximal to the zone of proliferating cartilage is the zone of hypertrophic cartilage, the cells of which appear swollen and their nuclei large and less deeply stained (zone 3, growing cells, Dodds and Cameron). The hypertrophic zone is normally sharply defined from the spongiosa.

This histological arrangement is equally apparent in healthy and in rachitic bones but, in the latter, the zones are wider, are often marred by degenerative



Fig. 2.—Tibia test fragment No. 85. Organic basal medium and aquasterol in equal parts. Uniform mineralization of hypertrophic zone. No mineralization of proliferative zone.

changes which may progress to actual cyst formation and to proliferative processes of osteoid tissue invading the demineralized spongiosa, the demarcation between which and the hypertrophic zone becomes irregular and ill-defined.

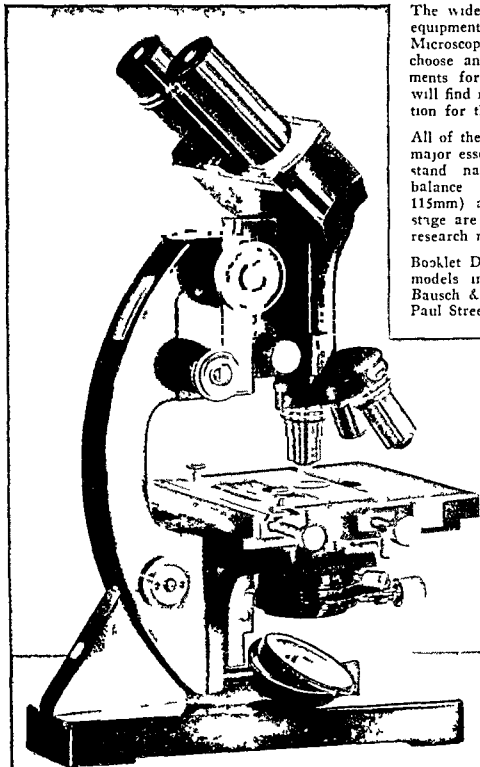
Most of our studies have been made on the proximal end of the tibia and our results are expressed in terms of the zones mineralized in that bone. We have included Figs. 1 to 3 as illustrations of the histological analysis upon which we have relied for the conclusions given in this paper.

#### EXPERIMENT NO. 1

Our first inquiry was the effect on mineralization of varying the amount of antirachitic principle in the immersion fluid. Experiment No. 1 (see Table),

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This and similar experiments (2, 3, 5, 6, 7, 8, 10, 11, 12) demonstrate that, in the presence of an adequate amount of utilizable calcium in the calcifying medium, the addition of aquasterol intensifies the extent and density of mineralization. Increase of the aquasterol content of the calcifying medium beyond the effective point does not produce a corresponding improvement of the mineralization.

Cystic formations in the hypertrophic zone, particularly at the limit of the spongiosa, and osteoid penetration from the spongiosa into the hypertrophic zone are both frequently seen in rachitic bones. These pathological features have no real significance in our study. They merely indicate that the rachitic process was already more extensive than the stage required for *in vitro* studies.

#### EXPERIMENT NO. 2

This experiment was devised to investigate the effect of increasing the vitamin D and reducing the calcium concentration in the calcifying medium.

Fragment 87, the negative control, showed no calcification. The positive control, fragment 88, showed calcification of poor density widely distributed throughout the hypertrophic zone but not extending into the proliferative zone. Fragment 89 was immersed in a medium of calcium concentration identical with that used for fragment 88, namely 0.025 gm. per 5.0 c.c., but using 3.8 c.c. aquasterol instead of 3.8 c.c. of water as diluent. The calcification in this fragment was denser and more uniform in the hypertrophic zone than in fragment 88 and extended to the limit but not into the substance of the proliferative zone as in fragments 85, 86 though lacking the density of mineralization characteristic of these fragments.

The significance of this experiment is that even in the presence of a diminished calcium concentration the extent and density of calcification in the growth area are favorably influenced by the presence of vitamin D in the calcifying medium but the density of mineralization depends ultimately upon the calcium concentration of the medium.

#### EXPERIMENT NO. 3

Experiment No. 3 duplicates in all details experiment No. 1 with precisely the same results. For this experiment fragments 91, 92, 93, 94 were used.

#### EXPERIMENT NO. 4

In order to rule out any possible effect of oil itself on mineral deposition we substituted for aquasterol the filtrate of an aqueous trituration of olive oil.

Fragment 95, the negative control, presents a severe degree of rickets. Spongiosa and cortex are very poorly mineralized and the hypertrophic zone is not merely devoid of calcification but has undergone considerable destruction through the proliferation of osteoid tissue.

Fragment 96, immersed in a calcifying bath identical with that described for fragment 84 (i.e. 0.05 gm. calcium glycerophosphate in 5 c.c. of calcifying medium), shows a widely distributed calcification of poor density extending throughout the zone of hypertrophic cartilage. The osteoid tissue invading this zone is also lightly mineralized. Cortex and spongiosa are, however, no denser than in fragment 95.

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## *CLINICAL AND EXPERIMENTAL*

### EXPERIMENTS ON THE MAINTENANCE OF MINERAL DENSITY IN THE SKELETON\*

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**I**N A series of experiments previously recorded<sup>9</sup> we have investigated the efficacy of aqueous preparations of the antirachitic principle in maintaining or restoring a healthy structural appearance in the growing ends of the long bones of rats fed on a mineral deficiency diet. Despite the restoration, these aqueous preparations, like the oleaginous preparations of vitamin D, were incapable of maintaining adequate mineralization of compacta and spongiosa of the shafts in the absence of a sufficient mineral intake. Owing to the fact of growth there results a progressive osteoporosis roentgenographically and histologically apparent. Ash values become relatively low and a generalized neuro-muscular irritability supervenes already described by Templin and Steenbock.<sup>8</sup>

Having learned that aqueous preparations are both possible and efficacious we turned to the mechanism of calcium deposit in the growing ends of bones, utilizing for our study *in vitro* methods in order to secure simplicity of control and eliminate the complications inseparable from tissue growth in the living body.

#### TECHNIC OF EXPERIMENTS

White rats, bred in the laboratory, were weaned at one month of age and immediately placed upon the rachitogenic diet No. 2965 of Steenbock and Black.<sup>6</sup> After one month on this feeding the animals were killed. The upper ends of both tibiae were dissected out under sterile conditions freed from adhering tissues and split longitudinally into halves. Thus each animal served for one

\*From the Anatomical Laboratory and Associated Foundations, Western Reserve University.

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Fragment 120, immersed in a medium of 2.5 c.c. volume containing calcium glycerophosphate 0.0075 gm., shows very imperfect mineralization in the hypertrophic zone alone, the mineral being distributed erratically and in varying density along the boundary line between hypertrophic and proliferative zones.

Fragment 119 was used as a negative control.

In the presence of a constant amount of vitamin D, a variation in the calcium of the medium results in a corresponding difference in extent and density of mineralization.

The foregoing experiments illustrate three significant features of bone mineralization.

1. The *in vitro* effect of aqueous preparations of vitamin D in favorably affecting the mineralization cannot be explained by any physical action of inert oil.

2. In the presence of a constant amount of vitamin D, variation in total available calcium results in a corresponding difference in extent and density of mineralization.

3. In the presence of a constant total available calcium the increase of vitamin D beyond a certain definite level gives no corresponding increase in extent or density of mineralization.

To amplify and confirm the observations recorded in these experiments just set forth the following supplemental experiments were carried out.

Experiment 7 doubles the maximum total amount of calcium glycerophosphate utilized in experiment 6. The fragments used were Nos. 123, 124, 125. Fragment 125 shows a far heavier mineralization in the presence of aquasterol than fragment 124 which was immersed in the glycerophosphate solution diluted with water alone.

Experiment 8, utilizing fragments 140, 141, 142, supplements experiment 7 by doubling the amount of aquasterol, the calcium glycerophosphate in the medium being maintained at a level intermediate between those of experiments 6 and 7. The result is identical in principle with that of experiments 6 and 7.

Experiment 9 duplicates experiment 8 but utilizes an aqueous preparation of an official U.S.P. cod liver oil in place of aquasterol. The fragments employed were Nos. 155, 156, 157. No. 157 shows a heavier mineralization than the positive control No. 156. This result should be contrasted with that of the olive oil experiment No. 4.

Experiment 10 supplements experiment No. 2, using approximately one fourth the amount of aquasterol. Fragments 171, 172, 173 were employed. The results are identical in principle with those of experiment 2.

Experiment 11 duplicates experiment 6 but uses a slightly greater amount of aquasterol and a smaller amount of calcium. Fragments used were Nos. 175, 176, 177, 178. The results are identical in principle with those of experiment 6.

Experiment 12, using fragments 179, 180, 181, supplements experiments 7 and 8. This is intermediate in calcium content (i.e., volume of calcifying medium) between experiments 9 and 10 and is almost identical in calcium content with experiments 1 and 3. The results simply confirm those already stated.

calcium glycerophosphate as is noted by percentage in the table. The concentration of the immersion bath was regulated by diluting the calcifying medium with sterile distilled water. To study the effect of vitamin D our aquasterol preparation was used as the diluent in place of distilled water. The experiments consisted in varying the amount of aquasterol and the calcium concentration in the immersion bath.

Assessments of the mineralization both in test material and controls were made upon histological sections prepared as already set forth.<sup>2</sup>

At first arbitrary ratings from 0 to 60 were devised on the basis of, but not identical with, Dyer's histological standards.<sup>3</sup> Rating No. 10 identifies the



Fig. 1—Experiment 1. Positive control. Tibia fragment No. 84. Organic basal (calcifying) medium without aquasterol. A practically unbroken cap of mineralization between spongiosa and hypertrophic zone of cartilage. Small irregular isolated areas of mineralization in hypertrophic zone itself.

average amount of restoration characteristic of immersion in the calcifying non-aquasterol containing medium of the positive control experiment. Rating No. 60 represents the calcification comparable with that of a normal bone, for aquasterol preparations never cause encroachment of mineralization into the diaphyseal epiphyseal plane. Graded valuations were assigned to intermediate stages of mineralization. Further experience has shown that the method of evaluation, while important in the biological assay of vitamin D preparations, does not permit the fine discrimination necessary for this study. The method which has given us greatest satisfaction is a histological analysis. The growing

## SUMMARY

1. Experiments 1, 4 and 9 recorded in the body of this paper illustrate the following points.

Aqueous preparations of vitamin D favorably influence the extent and density of mineralization in rachitic bone fragments immersed in a solution of organic calcium.

This effect cannot be attributed to surface tension or other physical properties of oil present in our aqueous preparations of vitamin D.

2. The remaining experiments demonstrate two further principles.

In the presence of a constant amount of vitamin D, variation of the organic calcium concentration results in a corresponding effect on extent and density of mineralization in the growing area.

In the presence of a constant organic calcium concentration in the calcifying medium a variation of vitamin D content does not produce corresponding effect on mineralization of the growing area.

3. In surveying the results of these experiments it is essential to keep in mind the fact that the part of bone under observation is the growing area only. The epiphysis proper and the shaft are not studied in this communication. Results evident in the growing area must not be presumed to hold for shaft or epiphysis. For these areas further specific studies must be designed.

## REFERENCES

1. Carlton, H. M., and Haynes, F.: *Histological Technique*, London, Oxford Univ. Press, p. 164, 1926.
2. Dodds, G. S., and Cameron, H. C.: Structural Modifications of the Epiphyseal Cartilages in the Tibia and Other Bones, *Am. J. Anat.* 55: 135, 1934.
3. Dyer, F. J.: The Standardization of Vitamin D by the Line Test, *Quart. J. Pharm. & Pharmacol.* 4: 503, 1931.
4. Kramer, B., Shelling, D. H., and Orent, E. R.: Studies Upon Calcification in Vitro: I. Effect of Reaction on Calcification, *Proc. Soc. Exper. Biol. & Med.* 24: 240, 1926.
5. Robison, R., and Soames, K. M.: The Possible Significance of Hexosephosphoric Esters in Ossification, VIII. Calcification in Vitro, *Biochem. J.* 24: 1922 (supp. 1923), 1930.
6. Steenbock, H., and Black, A.: Fat Soluble Vitamins. XXIII. The Induction of Growth Promoting and Calcifying Properties in Fats and Their Unsaponifiable Constituents by Exposure to Light, *J. Biol. Chem.* 64: 263 (see p. 274), 1925.
7. Shipley, P. G., Kramer, B., and Howland, J.: Studies Upon Calcification *in vitro*, *Biochem. J.* 20: 379, 1926.
8. Templin, V. M., and Steenbock, H.: Vitamin D and the Conservation of Calcium, *J. Biol. Chem.* 100: 209, 1933.
9. Venar, Y., and Todd, T. W.: The Efficacy of Vitamin D Administration in Aqueous Preparation, *J. Nutrition* 8: 553, 1934.

utilizing fragments Nos 83, 84, 85, 86, maintains in the calcifying bath a uniform concentration of calcium glycerophosphate (0.05 gm in 5 c.c.) for all fragments except the negative control No 83 which was immersed in distilled water. Fragment 84 was immersed in the organic basal solution diluted with water. For fragments 85 and 86 the diluent was aquasterol instead of distilled water but the amount of aquasterol used for No 85 was two thirds that used for No 86.

In the negative control (No 83) the mineralization of the fragment extends, however inadequately, through the spongiosa of shaft but does not penetrate the zone of hypertrophic cartilage. In the positive control (No 84) the spongiosa



Fig 3—Tibia test fragment No 86. Organic basal medium and aquasterol in proportions 1:3. Mineralization precisely as in Fig 2 despite greater content of aquasterol.

may be more densely mineralized but the characteristic features are a practically unbroken cap of mineralization between spongiosa and the zone of hypertrophic cartilage, and secondly small irregular isolated areas of mineralization in the hypertrophic zone itself (Fig 1).

In both test fragments (Nos 85, 86) not only is there a denser mineralization of spongiosa but the zone of hypertrophic cartilage is mineralized uniformly throughout its extent and the unaffected zone of proliferative cartilage together with the diaphyseal epiphyseal plane (zone of parallel rows of cartilage cells) remains as a narrow band clear of mineral deposit. Moreover there is no appreciable difference in the degree of mineralization in these two fragments despite the difference in aquasterol concentration (Figs 2 and 3).

It was soluble in 95 per cent alcohol, ether, petroleum ether chloroform, and only slightly soluble in methyl alcohol. This concentrate, when administered orally to animals having the granulocytopenic condition, showed marked granulocytopoietic activity.

The results are shown in Table I.

TABLE I  
RESULTS ON P-1

RABBITS	DATES	CONDITION	SUBSTANCE ADMINISTERED	TOTAL LEUCOCYTES	POLYNUCLEAR NEUTROPHILES	POLYNUCLEAR NEUTROPHILES, DEGENERATED	MONOCYTES	TRANSITIONAL CELLS	BASOPHILES	EOSINOPHILES	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES
1	4/ 9/35	*N	-	8,700	52	-	2	2	1	-	38	5
	4/ 9/35	O	-	-	-	-	-	-	-	-	-	-
	4/10/35	PO	-	5,100	28	2	2	-	-	-	60	8
	4/11/35	PO	5 c.c. P-1	2,900	20	-	3	1	-	-	67	9
	4/12/35	PO	-	8,900	50	-	4	2	1	-	37	6
	4/13/35	PO	5 c.c. P-1	9,150	8	2	1	-	1	-	80	8
	4/15/35	PO	5 c.c. P-1	8,500	45	-	2	-	-	1	45	7
	4/16/35	PO	5 c.c. P-1	9,700	51	-	3	1	2	-	33	10
	4/17/35	PO	5 c.c. P-1	9,000	50	-	4	-	-	-	40	6
		D										
2	4/ 9/35	N	-	8,750	47	-	6	1	1	-	32	13
	4/ 9/35	O	-	-	-	-	-	-	-	-	-	-
	4/10/35	PO	-	7,200	16	-	-	1	1	-	71	11
	4/11/35	PO	5 c.c. P-1	8,750	16	-	3	-	1	1	70	9
	4/12/35	PO	5 c.c. P-1	15,500	57	-	-	3	-	-	28	12
	4/13/35	PO	5 c.c. P-1	9,050	48	-	4	-	1	-	39	8
	4/15/35	PO	5 c.c. P-1	9,000	50	-	3	2	2	1	36	6
		D										
3	4/ 9/35	*N	-	8,700	46	-	2	1	-	1	40	10
	4/10/35	O	-	-	-	-	-	-	-	-	-	-
	4/12/35	PO	-	10,000	30	8	1	-	1	-	45	15
	4/13/35	PO	-	9,050	33	3	4	-	-	-	53	7
	4/13/35	PO	5 c.c. P-1	5,100	7	-	1	-	-	-	82	10
	4/15/35	PO	5 c.c. P-1	8,900	40	5	-	3	1	1	41	9
	4/16/35	PO	5 c.c. P-1	7,500	51	-	3	1	-	1	34	10
	4/17/35	PO	5 c.c. P-1	9,000	56	-	1	2	1	-	27	13
	4/18/35	PO	5 c.c. P-1	9,300	49	-	5	-	1	1	40	4
		D										
4	5/ 6/35	N	-	9,350	45	-	1	3	2	-	46	3
	5/ 7/35	N	-	9,950	50	-	3	1	1	-	42	3
	5/ 8/35	O	-	-	-	-	-	-	-	-	-	-
	5/ 9/35	PO	-	4,050	17	9	1	-	-	-	65	8
	5/10/35	PO	10 c.c. P-1	2,500	4	10	2	3	-	-	70	11
	5/10/35	PO	-	6,000	40	2	4	1	1	-	45	7
	P.M.											
	5/11/35	PO	10 c.c. P-1	4,550	19	-	1	3	2	-	59	16
	5/13/35	PO	10 c.c. P-1	8,500	47	-	2	-	-	-	41	10
	5/14/35	PO	10 c.c. P-1	9,000	52	-	1	1	1	-	40	5
	5/15/35	PO	10 c.c. P-1	7,050	48	-	3	-	-	1	38	10
	5/17/35	PO	10 c.c. P-1	8,600	50	-	6	-	-	1	37	6
		D										

\*N, normal; O, operated; PO, postoperative; D, discontinued.

*Preparation 2 (P-2).*—Two kilograms of bone marrow were saponified exactly as described under Preparation 1. One liter of distilled water was added

Fragment 97 was immersed in the same medium as fragment 96 but the diluent used was the aqueous olive oil preparation instead of distilled water in the proportion one half calcifying medium to one half aqueous olive oil. The histological features of this preparation are identical with those of No. 96.

Fragment 98 was immersed in a similar medium of identical calcium concentration as fragments 96 and 97 but the amount of aqueous olive oil was increased to 3.8 cc in a total of 5.0 cc. The calcium glycerophosphate content of the 5.0 cc was 0.05 gm as for Nos. 96, 97. No calcification took place.

From these experiments therefore it is evident that olive oil, in itself, is without favorable effect on mineralization.

Aqueous titrations of cod liver oil prepared in the same manner show if anything a slightly greater mineralization than the corresponding positive control employing the organic basal solution alone (see Experiment No. 9).

#### EXPERIMENT NO. 5

This experiment, utilizing fragments 99, 100, 101, 102, was performed for determining the effect of minimal concentrations of aquasterol in the presence of a slightly increased calcium concentration.

Fragment 99 (negative control) shows no calcium deposit even in the hypertrophic zone. Fragment 100 (positive control), immersed in 5.0 cc of the calcifying medium containing 0.75 gm of calcium glycerophosphate, shows a well mineralized hypertrophic zone up to but not into the proliferative zone. Fragment 101, immersed in a medium of nearly the same calcium concentration to which was added 1.00 cc of aquasterol (6.0 cc total volume equal to 0.075 gm calcium glycerophosphate), shows much denser mineralization extending throughout the hypertrophic zone clear to the limit of the proliferative zone. Fragment 102, immersed in equal parts of the basal solution and aquasterol (5.0 cc containing 0.03 gm calcium glycerophosphate), shows a mineralization equally extensive and of approximately equal density with fragment 101, though of definitely greater density than fragment 100.

Thus the presence of vitamin D, even in small amounts, enhances both the extent and density of mineralization but the presence of vitamin D in larger amounts will not substitute for a deficiency of calcium in the immersing medium.

That this is also evident in experiments on living animals was shown in a previous communication.<sup>9</sup>

#### EXPERIMENT NO. 6

In experiment No. 6 the aquasterol content was kept constant but the calcium concentration was diminished. For this experiment fragments 119, 120, 121, 122 were used. Fragment 122 was immersed in a medium of 4.0 cc volume containing 0.03 gm of calcium glycerophosphate. This concentration is greater than that used for fragments 89 (experiment No. 2) and 102 (experiment No. 5) and gave practically identical results.

Fragment 121 was placed in a medium of 3.0 cc volume containing 0.015 gm of calcium glycerophosphate. Mineralization is sparsely distributed throughout the hypertrophic zone and does not penetrate the zone of proliferative cartilage.

to the saponifying solution, and after shaking thoroughly, 1 liter portions were extracted with 500 c.c. of petroleum ether (boiling point 40° to 50° C.). After the ether layer separated out, the underlying soap solution was drawn off. When all the saponifying solution was thus extracted, the petroleum ether solution was resaponified with 3 liters of 3 per cent alcoholic potassium hydroxide at room temperature for twenty-four hours. The soap formed by this procedure was insoluble in the liquid medium and was easily separated by filtration aided by suction. The soap was washed 3 times with 50 c.c. portions of petroleum ether. The addition of 1 liter of distilled water to the filtrate caused the formation of 2 layers. The upper layer was saved and the lower discarded. One liter portions of the petroleum ether solution were washed 5 times with 100 c.c. of 40 per cent alcohol; when all of the ether solution was thus washed, it was collected and distilled off under diminished pressure at 30° C. The resulting product had a slight cloudiness, but after filtering through a layer of asbestos it remained clear. Twenty grams of the concentrate were obtained from the original 2 kg. of bone marrow.

This concentrate was a brilliant amber, oily liquid, being odorless and imparting a faint oily taste. The specific gravity was 0.8276. It gave a neutral reaction to phenolphthalein, a negative test for sterols, and it was soluble in 95 per cent alcohol, ether, petroleum ether and chloroform. When this substance was administered intramuscularly to animals which showed a condition of granulocytopenia, a marked granulocytopoietic activity was observed within four hours.

The results are given in Table II.

*Etiology of Granulocytopenia.*—Since the disease granulocytopenia was first described in Germany in 1922<sup>5</sup> and in the United States in 1924,<sup>6</sup> many efforts<sup>6-20</sup> have been made to determine its cause. Many theories of the etiology of this disease have been advanced, namely, dietary deficiency,<sup>7, 8</sup> hormonal dysfunction,<sup>9-12</sup> use of certain coal-tar derivatives,<sup>13-17</sup> and bacterial infection.<sup>18-20</sup>

#### METHODS OF PRODUCING GRANULOCYTOPENIC CONDITIONS EXPERIMENTALLY

1. *Chemical Method.*—Granulocytopenic conditions could not be produced in rabbits and rats by the administration of hydroquinone, amidopyrine, or benzene.

2. *Bacterial Method.*—The procedure used was a modification of the method described by Dennis.<sup>18</sup> Ten cubic centimeters of a twenty-four-hour culture of *Staphylococcus aureus* were placed into a Naturalamb skin sac which had been moistened with distilled water. The open end of the sac was sealed by tying with a linen thread. The outer surface of the sac was sterilized by immersing several times in 70 per cent alcohol.

The normal total and differential leucocytic counts were determined on each animal. The rabbits were starved for twenty hours before the operation. A light ether anesthesia was administered, and the peritoneal cavity was exposed by incision, care being exercised to maintain aseptic conditions. A sac, prepared as described above, was placed into the lower part of the peritoneal cavity. The peritoneum and abdominal wall were sutured with plain catgut. Metal clips were used on the skin. A bandage was applied and held in position by a

TABLE I  
OUTLINE OF DATA UPON THE TWELVE EXPERIMENTS RECORDED IN THIS PAPER

EXPERIMENT NO	FRAGMENT NO	TOTAL VOL BATH CC	VOLUME CALCIFYING MEDIUM CC	VOLUME WATER CC	VOLUME EXP SOLUTION CC	PER CENTAGE GLYCEROL PHOSPHATE	RATING ON CALCIFICATION
1	83	50		50		0	0
	84	50	25	25		10	5
	85	50	25		25	10	50
	86	50	12		38	10	50
2	87	50		50		0	0
	88	50	12	38		05	20
	89	50	12		38	05	45
3	91	50		50		0	0
	92	50	25	25		10	5
	93	50	25		25	10	40
	94	50	12		38	10	40
4	95	50		50		0	0
	96	50	25	25		10	25
	97	50	25		25	10	25
	98	50	12		38	10	5
5	99	50		50		0	0
	100	50	50			15	40
	101	60	50		10	127	60
	102	50	25		25	15	60
6	119	20			20	0	0
	120	25	05		20	03	10
	121	30	10		20	05	25
	122	40	20		20	075	35
7	123	50		50		0	0
	124	50	40	10		12	40
	125	50	40		10	12	60
8	140	60		60		0	0
	141	60	40	20		10	30
	142	60	40		20	10	50
9	155	60		60		0	0
	156	60	40	20		10	30
	157	60	40		20	10	50
10	171	30		30		0	0
	172	30	20	10		10	30
	173	30	20		10	10	50
11	175	30		30		0	0
	176	30	05	25		025	15
	177	30	05		25	025	25
	178	30	025		275	0125	5
12	179	50		50		0	0
	180	50	30	20		09	40
	181	50	30		20	09	50

In all experiments except Nos 4 and 9 the experimental solution is aquasterol prepared in the manner previously described.

In Experiment 4 the experimental solution is olive oil in No 9 it is a standard U S P cod liver oil

fractions which appear to possess granulocytopoietic properties. As described under Preparations 1 and 2, the active fractions were isolated by two methods, namely, by separation with distilled water and by petroleum ether extraction. The former method affords a comparatively simple means of separation. However, by the latter method it is possible to obtain the fraction in a more concentrated form. In addition, the foregoing data show that Preparation 1 is active when administered orally, and that Preparation 2 is active when administered intramuscularly to rabbits in which a condition of granulocytopenia was produced experimentally. Although a very highly active concentrate was prepared, it will be necessary to conduct further studies on the purification and concentration, before any suggestion of chemical composition can be made.

In this work, attempts to produce granulocytopenic conditions in rabbits and rats by the use of hydroquinone, amidopyrine, or benzene were unsuccessful. Consequently, a modification of the method employed by Dennis<sup>18</sup> was used and proved to be satisfactory. Although the condition obtained by this procedure may not be identical with that in human beings, it affords a convenient laboratory method of demonstrating the granulocytopoietic activity of yellow bone marrow concentrates.

#### CONCLUSION

Highly concentrated fractions, possessing granulocytopoietic activity, were prepared from yellow bone marrow.

I wish to express my thanks to Dr. C. A. Hoppert and Dr. C. W. Geiter for their interest in this work and for their helpful advice and criticism.

#### REFERENCES

1. Minter, M. M.: The Treatment of Agranulocytic Angina, *Southwest Texas Med.* 2: 8, 1935.
2. Harris, W. H., and Schattenberg, H. J.: So-Called Agranulocytic Angina With Special Consideration of the Causal Agent, *New Orleans M. & S. J.* 88: 238, 1935.
3. Burcky, F. W.: Agranulocytosis, *Illinois M. J.* 67: 59, 1935.
4. Watkins, C. W.: Personal communication to the author in November, 1934.
5. Schultz, W.: Ueber eigenartige Halserkrankungen, *Deutsche med. Wchnschr.* 48: 1494, 1922.
6. Lovett, B. R.: Agranulocytic Angina, *J. A. M. A.* 83: 1498, 1924.
7. Langston, W. C., and Day, P.: Cited by Kracke, R. R., and Parker, F. P.: The Relationship of Drug Therapy to Agranulocytosis, *J. A. M. A.* 105: 900, 1935.
8. Miller, D. D., and Rhoads, C. P.: Experimental Production in Dogs of Acute Stomatitis, Associated With Leukopenia and a Maturation Defect of the Myeloid Elements of the Bone Marrow, *J. Exper. Med.* 61: 173, 1935.
9. Britton, S. W., and Corey, E. L.: Blood Cellular Changes in Adrenal Insufficiency and the Effects of Cortico-Adrenal Extract, *Am. J. Physiol.* 102: 699, 1932.
10. Thompson, W. P.: Observations on the Possible Relation Between Agranulocytosis and Menstruation, With Further Studies on a Case of Cyclic Neutropenia, *New England J. Med.* 201: 176, 1934.
11. Jackson, H., Jr., Merrill, D., and Duane, M.: Agranulocytic Angina Associated With Menstrual Cycle, *New England J. Med.* 210: 176, 1934.
12. Rutledge, B. H., Hansen-Pruss, O. C., and Thayer, W. S.: Recurrent Agranulocytosis, *Bull. Johns Hopkins Hosp.* 46: 369, 1930.
13. Weiskotten, H. G.: The Action of Benzol: The Normal Life Span of Neutrophile (Amphophile) Leukocyte (Rabbit), *Am. J. Path.* 6: 183, 1930.
14. Kracke, R. R.: The Experimental Production of Agranulocytosis, *Am. J. Clin. Path.* 2: 11, 1932.
15. Madison, F. W., and Squier, T. L.: The Etiology of Primary Granulocytopenia, *J. A. M. A.* 102: 755, 1934.

# GRANULOCYTOPOIETIC FRACTIONS OF YELLOW BONE MARROW\*

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IN RECENT years the condition known as granulocytopenia has attained a prominent position in the medical world. While the etiology of the disease still remains obscure, the many agents employed for its treatment have met with varying degrees of success.<sup>1, 2</sup> Of these therapeutic agents, yellow bone marrow has attracted much attention.

Watkins<sup>4</sup> has obtained encouraging results by oral administration of bone marrow at the onset of the disease. He gave daily doses of approximately 60 to 120 gm of fresh yellow bone marrow, obtained from beef, until the blood assumed a normal picture, and smaller doses for several months later. Since large amounts of marrow were necessary to obtain a change in the leucocytic picture, and since in certain cases the patient was unable to digest and assimilate such large quantities, the use of the fresh marrow for this purpose was considered undesirable. Accordingly, a study was undertaken to separate the granulocytopoietic fraction from yellow bone marrow, and to determine its activity on experimental animals.

## EXPERIMENTAL

*Source of Bone Marrow*—The bone marrow used in these studies was obtained from fresh long bones of cattle. It was freed of blood, bony and fibrous material and then ground.

*Preparation 1 (P 1)*—Two kilograms of bone marrow were saponified with 6 liters of 3 per cent alcoholic potassium hydroxide at room temperature for twenty four hours. The saponification was aided by mixing the solution with a mechanical stirrer. At the end of twenty four hours the solution was filtered through a large fluted filter. The residue consisted chiefly of insoluble bony and fibrous tissues. An equal volume of distilled water was added to the filtrate which separated into two layers. The upper layer containing the nonsaponifiable fraction was saved and the lower soapy layer discarded. In order to free the concentrate from alkali, it was washed 5 times with 50 cc of distilled water and 5 times with 50 cc of 40 per cent alcohol. From the original 2 kg of bone marrow, 100 gm of the concentrate were obtained.

The product thus obtained was a yellow, oily liquid being nearly odorless and having a slightly oily after taste. It had a neutral reaction to phenolphthalein, gave a negative test for sterols, and had a specific gravity of 0.8626.

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suggesting the red cells exert a sort of buffer action. Ether anesthesia<sup>7</sup> affects chiefly the red cells. Boyd and Wilson<sup>8</sup> have shown that lipids are carried by umbilical blood from the placenta to the fetus; in unpublished experiments, the author has found that this transport may be effected in part by the red blood cells. Last it may be noted that following parturition in women there sets in a momentary increase in the lipid content of the red cells.<sup>5</sup>

The lipid content of the red cells may be estimated directly by removing the cells from below the supernatant plasma after centrifuging the blood or indirectly from concurrent analyses of whole blood and plasma in conjunction with the hematocrit reading. Both of these methods are at present employed and frequently the results of one investigator using the direct method have been compared with those of another using the indirect. Hence, it was desirable to know which of these methods is the more exact and whether they gave different results.

The technic of the indirect consists simply in making the usual Bloor extract or other common extract of both whole blood and plasma. The technic of the direct method has been less thoroughly described. In doing direct analyses, the sample of oxalated blood is thoroughly centrifuged for about three-quarters of an hour or until the red cell layer has become translucent. This indicates that the interstices between cells have been displaced and that the cells are squarely packed and contain little or no plasma. Two cubic centimeters of these cells are pipetted off with suction if necessary and transferred to a 125 c.c. Erlenmeyer flask and the cells laked with a minimum amount of distilled water (about 1 c.c.). Then about 60 c.c. of alcohol-ether (3:1) are quickly poured in and the whole immediately and thoroughly shaken. The flask may have to be shaken again at short intervals to prevent clumping of the precipitated proteins. If, in spite of this, clumping occurs, the adherent mass must be removed, ground in a cleaned mortar with cleaned, sharp sand and then quantitatively transferred back to the original flask. Extraction is incomplete unless the precipitate is finely divided. The remainder of the extraction and analysis may be performed as previously described for plasma.<sup>2, 4</sup>

In this manner the lipid composition of the red blood cells was determined directly and indirectly in 10 normal men, collecting the specimens of blood under fasting conditions. The results obtained have been depicted in Table I in the form of means with standard deviations. The standard deviation was calculated by means of a formula previously used.<sup>2</sup> The percentage standard deviation was calculated by dividing the standard deviation by the mean and multiplying by 100. To conserve space, individual values will be summarily described rather than tabulated.

With almost every lipid the results were higher by the indirect than by the direct method. In 8 out of 10 cases neutral fat was higher, in 7 out of 10 the total fatty acids were higher, in 6 out of 10 the total cholesterol was higher, in 5 out of 10 the ester cholesterol was higher, in 7 out of 10 free cholesterol was higher, and in 6 out of 10 phospholipid was higher. In most of the cases where the results were lower by the indirect method the differences were slight, while when they were higher they were usually considerably so. As a result practically all the mean values were higher by the indirect than by

TABLE II  
RESULTS ON P 2

RABBITS	DATES	CONDITION	SUBSTANCE ADMINISTERED	TOTAL LEUCOCYTES	LOI MYELOBLASTIC NEUTROPHILS	LOI MYELOBLASTIC MONONUCLEAR NEUTROPHILS	MONOCYTES	TRANSITIONAL CELLS	BASOPHILS	EOSINOPHILS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES
5	8/ 6/35	*N	-	7,200	49	-	4	1	1	1	31	13
	8/ 7/35	O	-	-	-	-	-	-	-	-	-	-
	8/ 8/35	PO	01 cc P 2	31,000	1	23	5	1	-	-	67	3
	8/ 8/35	PO	-	10,800	-	-	-	-	-	-	-	-
	8/ 9/35	PO	01 cc P 2	13,830	41	8	6	1	2	-	39	3
	8/12/35	PO	01 cc P 2	30,950	28	1	1	2	1	-	66	1
	8/13/35	PO	01 cc P 2	8,900	52	-	2	1	2	-	36	7
	8/14/35	PO	01 cc P 2	8,000	48	-	2	-	-	-	37	12
	8/15/35	PO	01 cc P 2	9,500	50	-	2	2	1	-	35	10
	8/16/35	PO	-	-	-	-	-	-	-	1	42	4
	8/19/35	PO	-	9,000	49	-	3	1	1	-	35	11
6	8/26/35	N	-	7,500	43	-	5	1	1	-	40	10
	8/26/35	O	-	-	-	-	-	-	-	-	-	-
	8/27/35	PO	-	13,400	43	5	1	-	-	1	48	2
	8/28/35	PO	05 cc P 2	3,600	4	8	2	-	2	-	71	12
	8/29/35	PO	05 cc P 2	17,550	42	-	2	-	4	-	42	10
	8/30/35	PO	05 cc P 2	7,700	54	-	5	3	5	-	28	5
	9/ 3/35	PO	05 cc P 2	8,000	53	-	3	1	1	-	34	8
		D	-	-	-	-	-	-	-	-	-	-
	10/ 2/35	N	-	9,400	52	-	2	1	-	1	37	7
	10/ 2/35	O	-	-	-	-	-	-	-	-	-	-
7	10/ 3/35	PO	-	6,800	12	59	5	1	-	-	16	7
	10/ 4/35	PO	01 cc P 2	4,000	-	25	3	-	-	-	67	5
	10/ 5/35	PO	01 cc P 2	9,350	48	24	-	-	-	1	22	5
	10/ 6/35	PO	01 cc P 2	10,750	54	4	7	2	2	-	25	6
	10/ 7/35	PO	01 cc P 2	9,000	51	-	5	3	1	1	30	9
	10/ 8/35	PO	-	7,500	48	-	3	1	-	-	38	10
	10/ 9/35	D	-	-	-	-	-	-	-	-	-	-
	10/ 2/35	*N	-	6,800	48	-	4	1	1	1	33	12
	10/ 2/35	O	-	-	-	-	-	-	-	-	-	-
	10/ 3/35	PO	-	6,500	7	58	-	-	3	-	32	-
8	10/ 3/35	PO	-	6,150	1	23	4	1	1	-	66	4
	10/ 4/35	PO	01 cc P 2	3,900	4	12	-	-	-	-	80	4
	10/ 4/35	PO	01 cc P 2	6,800	37	2	3	3	1	2	48	4
	10/ 5/35	PO	01 cc P 2	6,350	52	-	3	1	3	1	35	5
	10/ 6/35	PO	01 cc P 2	7,200	43	-	-	-	2	-	47	8
	10/ 7/35	PO	-	8,500	50	-	1	1	1	1	37	9
	10/ 8/35	D	-	-	-	-	-	-	-	-	-	-
	10/ 2/35	N	-	8,900	53	-	2	3	-	1	34	7
	10/ 2/35	O	-	-	-	-	-	-	-	-	-	-
	10/ 3/35	PO	-	7,700	21	12	2	-	1	-	50	14
9	10/ 4/35	PO	-	21,900	6	29	3	-	1	-	57	4
	10/ 4/35	PO	01 cc P 2	14,250	4	6	-	-	3	-	80	7
	10/ 5/35	PO	01 cc P 2	9,500	15	5	2	2	1	1	70	4
	10/ 6/35	PO	01 cc P 2	8,150	20	5	4	1	-	-	64	6
	10/ 7/35	PO	01 cc P 2	8,000	48	1	3	-	2	-	36	10
	10/ 8/35	PO	-	7,200	46	-	2	4	-	-	43	5
	10/ 9/35	PO	-	9,100	49	-	-	1	2	1	36	11

\*N, normal, O, operated, PO, postoperative D discontinued

3. Boyd, E. M.: The Lipemia of Pregnancy, *J. Clin. Investigation* 13: 347, 1934.
4. Boyd, E. M.: Diurnal Variations in Plasma Lipids, *J. Biol. Chem.* 110: 61, 1935.
5. Boyd, E. M.: Blood Lipids in the Puerperium, *Am. J. Obst. & Gynec.* 29: 797, 1935.
6. Boyd, E. M.: The Lipopenia of Fever, *Canadian M. A. J.* 32: 500, 1935.
7. Boyd, E. M.: Anaesthesia and the Blood Lipids, *Surg. Gynec. Obst.* 62: 677, 1936.
8. Boyd, E. M.: The Effect of Pregnancy and Pseudopregnancy Upon the Blood Lipoids of Rabbits, *J. Physiol.* 86: 250, 1936.
9. Boyd, E. M., and Wilson, K. M.: The Exchange of Lipids in the Umbilical Circulation at Birth, *J. Clin. Investigation* 14: 7, 1935.
10. Boyd, E. M., and Tweddell, H. J.: The Lipids of Human Blood, *Trans. Royal Soc. Canada, Section V* 29: 113, 1935.
11. Boyd, E. M., and Fellows, M.: Blood Lipids During Pregnancy in Guinea Pigs, *Am. J. Physiol.* 114: 635, 1936.
12. Wendt, H.: Relation Between Phosphatides and Cholesterol in Whole Blood, Plasma and Erythrocytes of Healthy Men Overdosed With Olive Oil With and Without Previous Phosphate Administration, *Biochem. Ztschr.* 250: 212, 1932; *ref. Chem. Abstr.* 26: 5139, 1932.

## BLOOD CHOLESTEROL AND THE MANIC DEPRESSIVE PSYCHOSIS\*

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CHOLESTEROL is one of the most important constituents of cells, particularly cells of the central nervous system. It is important because it can, and does, exert much influence upon cellular metabolism, and, therefore, upon body metabolism in general. Although it has received much attention in many physiologic and pathologic conditions, it has been grossly ignored and neglected in mental disease. This is rather astonishing when one considers the fact that nervous tissue possesses an abundance of this chemical substance and that exclusive of its water content the brain is composed of about one-half lipoids of which over 25 per cent is cholesterol. With cholesterol composing the brain to this extent, neglect of its study, particularly in relation to mental disease, is almost heresy.

This paper is the preliminary report of a study of the blood cholesterol in one type of mental disease, the manic depressive psychosis.

In it will be considered the relationship existing between this chemical substance and the manic depressive psychosis, the manic phase, the depressive phase, and the state of mental and physical activity of the individual.

### SURVEY OF LITERATURE

Pighini<sup>1</sup> studied seven cases of manic depressive psychosis and found the blood cholesterol to be increased. Tsuchiya<sup>2</sup> in his cases obtained only normal values. In the four cases of melancholia with anxiety examined by Targowla, Badonnel and Berman<sup>3</sup> the blood cholesterol was reported as high. Parhon and Parhon<sup>4</sup> stated that in their four cases of melancholia the values were below normal. In Ornstein's<sup>5</sup> cases the blood cholesterol was increased; in

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binder. Blood for the counts was obtained from the marginal vein of the ear, and the smears were stained by Wright's method.

The granulocytopoietic effect produced by this procedure was determined by making total and differential leucocytic counts. The results of these counts were used as the criterion of the effects produced on the granulocytopoietic system by the bone marrow concentrates.

Forty six rabbits were prepared according to this method. Of this number 17 developed a condition of granulocytopenia, 9 died without showing any change in the leucocytic picture, and 20 recovered without any apparent ill effects.

*Rabbits 1, 2, 3 and 4*—Rabbits 1 and 2 (males) and 3 and 4 (females) each weighing about 2.5 kg were prepared according to the method described. All developed the granulocytopenic condition and recovered upon daily oral administration of P 1.

The data are given in Table I.

*Note* In these Tables the cells termed as polynuclear neutrophiles, degenerated, were polynuclear neutrophiles characterized by disintegration of the cell membrane and cytoplasm (presence of vacuoles) and a distortion of the nucleus. These cells stained very poorly.

*Rabbits 5, 6, 7, 8, and 9*—Rabbits 5, 6 and 7 (females) and 8 and 9 (males) each weighing approximately 2.8 kg were prepared as described. Rabbits 5 and 9 did not develop a typical condition of granulocytopenia. The second day after the operation Rabbit 5 had a total leucocytic count of 31,000 and Rabbit 9, 21,900. In both cases only a few normal polynuclear neutrophiles could be found. The animals were given 0.1 cc of P 2 intramuscularly daily for six days. At the end of this period, the blood picture of each animal returned to normal, and they showed apparent recovery. Two days after the operation Rabbits 6, 7, and 8 developed the granulocytopenic condition. Rabbit 6 was given 0.5 cc and Rabbits 7 and 8, 0.1 cc of P 2 intramuscularly daily for four days. At the end of the four day period, the animals showed a normal blood picture and appeared to be in good health.

The data are shown in Table II.

*Observations on the Absorption and Local Reaction of P 2*—Twelve full grown rats and 3 rabbits were used. The right leg of each animal was shaved and the skin was cleansed thoroughly with soap and water. Under aseptic conditions 0.2 cc of P 2 was injected into the muscle of the leg daily for four days. Each injection was made at the same site.

The injections did not cause any local reaction as manifested by the absence of redness, swelling, and restlessness in the animals. Four days after the last injections were made the animals were killed, and upon autopsy the muscles into which the preparation was injected showed no gross abnormality. This indicates definitely that this substance is easily absorbed when injected into an animal intramuscularly.

#### DISCUSSION

From these studies it is evident that by saponifying yellow bone marrow with alcoholic potassium hydroxide at room temperature it is possible to isolate

evident that only slightly less than one-fourth (23.8 per cent) of a total of 71 manic depressive individuals had blood cholesterol values within normal limits. As a group the manic depressive cases, therefore, present rather overwhelming evidence of abnormal cholesterol metabolism.

TABLE I

DISTRIBUTION OF BLOOD CHOLESTEROL VALUES IN NORMAL, MANIC AND DEPRESSIVE CASES.  
INDIVIDUALS GROUPED IN 10 MG. INTERVALS

NORMAL VALUES	NO. CASES	MANIC VALUES	NO. CASES	DEPRESSIVE VALUES	NO. CASES
110-120	3	50-60	1	50-60	1
120	4	60	1	70	2
130	6	70	2	80	4
140	8	80	3	90	3
150	11	90	9	100	2
160	10	100	12	110	1
170	5	110	2	120	1
180	5	120	1	140	4
190	1	140	1	150	3
Total	53	160	1	160	3
			33	200	2
				210	2
				220	2
				240	1
				250	1
				260	1
				280	1
				300	1
				320	1
				330	1
				420	1
					38

If the manic (33 cases) and the depressive (38 cases) groups are studied separately a similar although definitely altered picture is seen. In the manic group 28 (84.8 per cent) of the cases have cholesterol values which are below the limits of normal, and 5 (15.1 per cent) cases within it, and 5 (15.1 per cent) cases above it. In the depressive group there are 12 (31.5 per cent) cases with blood cholesterol values below normal, 12 (31.5 per cent) cases within normal limits, and 14 (36.8 per cent) cases above normal limits. The median value for manic cases is 100.4 mg. and for the depressive, 150 mg. It is, therefore, obvious that in the manic state there is a definite tendency for the blood cholesterol values to be decreased and below normal, whereas in the depressive cases there exists a fairly balanced tendency for the values to be not only within normal limits but below and above it in the same proportion. Briefly, 84.8 per cent of the manic cases and 68.3 per cent of the depressive cases have abnormal blood cholesterol values. This, too, is evidence of abnormal metabolism of a chemical substance which constitutes approximately 25 per cent of the lipoids of the brain.

To complete the study the relationship between the blood cholesterol and the state of the mental and physical activity was examined (Table II). When the physical activity alone is considered, whether increased (36 cases) or

- 16 Hoffman, A M, Butt, E M, and Hickey, H G Neutropenia Following Amidopyrine Preliminary Report, J A M A 102 1213, 1934
- 17 Special Reports of the Council on Pharmacy and Chemistry, A M A The Relation of Amidopyrine and the Barbituric Acid Derivatives to Granulocytopenia, J A M A 102 2183, 1934
- 18 Dennis, E W Experimental Granulopenia Due to Bacterial Toxins Elaborated In Vivo, J Exper Med 57 993, 1933
- 19 Felson, J Bacillary Dysentery—Acute Fulminating Type With Marked Toxic Neutropenia, New York State J Med 35 1037, 1935
- 20 Sholl, C P Personal communication to the author in March, 1935

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## A COMPARISON OF THE DIRECT VERSUS THE INDIRECT METHOD OF ESTIMATING THE LIPID COMPOSITION OF THE RED BLOOD CELLS\*

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THE rôle of the red blood cells in lipid metabolism is a difficult one to evaluate. On the one hand there is evidence that the erythrocytes may play a prominent part, but evidence of a contradictory nature suggests that they are inert. The point of present practical importance is that the lipid content of the red blood cells is not the same as that of plasma or serum and that when changes occur in plasma or serum these same changes do not occur in the red blood cells. As a result the lipid analysis of whole blood alone may fail to reveal any marked abnormality when actually opposite changes of significance have taken place in the cells and plasma. In any complete investigation both the cells and plasma should be examined, if only one value is determined then plasma or serum is to be preferred to whole blood.

In a number of conditions the lipids of the red cells have been found to remain unaltered in spite of marked changes in plasma and on the basis of this information the cells have been considered to be inert in fat metabolism. This has been shown in the persistent lipemias of diabetes, pregnancy, and chronic hemorrhage.<sup>1</sup> Wendt<sup>12</sup> reported that a fat meal caused an increase in plasma lipids but not those of the erythrocytes. Boyd<sup>8</sup> and Boyd and Fellows<sup>11</sup> have presented data which demonstrates that although the lipid composition of plasma differs markedly between man, rabbits, and guinea pigs, yet the lipid composition of the red blood cells is the same in all three species.

In other investigations the red cells have been found to take a prominent part in lipid metabolism, and this information is sufficient to warrant their inclusion in any serious, complete study of the blood lipids. In the first place, Bloor<sup>1</sup> has shown that a copious meal of fat produces an increase in the cellular lipids. Boyd and Tweddell<sup>10</sup> found that the ordinary meals do not appreciably affect the lipids of the red cells but that certain curious changes occurred in the early morning hours between midnight and rising. In fever<sup>6</sup> the concentration of lipids in the erythrocytes varies oppositely to that of plasma,

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for the values to be above and in the normal range. These findings are in accordance with the observations where, in the manic depressive group as a whole the median is definitely low, in the manic group the median is definitely low, and in the depressive group the median indicates a range of values, low, normal, and high.

These results may be questioned on the basis of the possible existence of factors other than the psychosis which may have been present to alter the blood cholesterol. Since the literature indicates that the blood cholesterol is increased in manic depressive psychoses, an attempt was made to determine whether pathologic entities other than the psychosis were present in the manic depressive cases. The results of this part of the study were entirely negative. No relationship could be demonstrated between blood cholesterol and clinically observed body type (medians: Pyknic 105.7 mg.; asthenic 103.3 mg.; and athletic 110.0 mg.) or state of abnormal nourishment (medians: obese 110 mg.; and undernourished 120 mg.). Albumin, indican, or acetone in the urine of some of the individuals apparently had no significance that could be demonstrated relative to the cholesterol. No relationship could be established between abnormal blood cholesterol and hemoglobin, erythrocyte count, or leucocyte count; nor could any relationship be established correlating blood cholesterol and systolic, diastolic, or pulse pressures.

#### DISCUSSION

The cases of manic depressive psychosis presented in this study can be divided roughly into three groups, viz., those with blood cholesterol (a) below, (b) above, and (c) within normal limits. For the purpose of this paper the cases with a normal value can be ignored, except for the recognition of the fact that a certain number of cases (23.8 per cent) *do* have a blood cholesterol within normal limits. This leaves 76.2 per cent of the cases with abnormal values to be explained. This 76.2 per cent automatically subdivides itself into those cases with values above normal (19.6 per cent), and those with values below normal (56 per cent).

*Three-fourths of the cases of manic depressive psychosis have an abnormal blood cholesterol. And of these cases almost three-fourths have a blood cholesterol below normal.*

The number of cases with an abnormal blood cholesterol and the distribution of this abnormality can hardly be due to chance. Since they must have some significance, the question naturally arises as to what it is that produces the abnormal blood cholesterol in these cases. At best, the most that can be done to answer the question at the present is to examine the possible sources whereby an abnormal value is obtained.

An increase in the blood cholesterol may be caused by (a) chronic liver disease, (b) diabetes mellitus, (c) arteriosclerosis, (d) lipid nephrosis, (e) glomerulonephritis, and (f) pregnancy. Inasmuch as these conditions are not present in any of the cases, they cannot be held responsible.

A decrease in the blood cholesterol can be caused by (a) anemia, (b) inanition, (c) infection, and (d) acute hepatic disease. Since these conditions are not present in any of the cases they, too, cannot be held responsible.

the direct method. It is thus not permissible to compare relative lipid values, some done by the direct and some by the indirect method of analysis.

The direct method was finally adopted in preference to the indirect after a consideration of the percentage standard deviations as listed in Table I. It may be noted that the percentage standard deviations were higher, sometimes by as much as three times, with the indirect than with the direct method. This signified that there was much greater variation among values obtained by the indirect method, a fact which was quite apparent from tables listing the individual values. Since values obtained by the indirect method are calculated from at least three sets of analytical figures, the experimental error involved is probably quite sufficient to account for this variation.

Further evidence in support of the superiority of the direct method was the fact that several "impossible values" were obtained by the indirect method and none by the direct. That is to say, the sum of the values for free cholesterol and ester cholesterol was greater than that of total cholesterol in three instances by the indirect method. In two other instances the total fatty acid value was less than the calculated sum of the individual fatty acids. As a result of these considerations, it was concluded that the direct method is to be preferred in the determination of the lipid composition of the red blood cells. This conclusion applies not only to the lipids in toto but to any one individual lipid such as total cholesterol.

TABLE I

A. STATISTICAL COMPARISON OF VALUES FOR LIPIDS IN THE RED BLOOD CELLS AS DETERMINED BOTH BY THE DIRECT AND BY THE INDIRECT METHODS IN 10 NORMAL MEN  
(EXPRESSED IN MG PER 100 CC)

LIPID	MEAN OF 10 CASES		STANDARD DEVIATION		PERCENTAGE STANDARD DEVIATION	
	DIRECT METHOD	INDIRECT METHOD	DIRECT METHOD	INDIRECT METHOD	DIRECT METHOD	INDIRECT METHOD
Total fatty acids	275	297	37	117	14%	40%
Neutral fat	27	54	31	80	115%	148%
Total cholesterol	150	158	19	32	13%	20%
Ester cholesterol	28	27	20	23	71%	85%
Free cholesterol	124	138	18	28	14%	20%
Phospholipid	356	424	47	145	13%	34%

## SUMMARY

The lipid composition of the red blood cells of ten normal men was estimated directly and indirectly by oxidative micromethods. In the majority of instances higher values were obtained by the indirect than by the direct method. The experimental error was found much greater by the indirect method which also gave more mathematically impossible values. Hence it was concluded that the direct method is to be preferred in the determination of any one or all of the lipids present in the red blood cells and that results obtained by one method cannot be compared with those obtained by the other.

## REFERENCES

- 1 Bloor, W. R. Fat Assimilation, *J Biol Chem* 24: 447, 1916
- 2 Boyd, E. M. A Differential Lipid Analysis of Blood Plasma in Normal Young Women by Micro Oxidative Methods, *J Biol Chem* 101: 323, 1933

## ALBUMINURIA SOLARIS\*

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**A**MONG the various causes responsible for the anephritic albuminurias, there still are important sources hitherto unknown or unpublished.

Albuminuria, as is well known, is not identical with nephritis, the same as glycosuria is not identical with diabetes. Most of the anephritic albuminurias are innocuous in character and usually transient in appearance. It is well to point out the existence of an occasionally observed analbuminuric nephritis (per analogiam aglycosuric diabetes), in contrast to the anephritic albuminuria.

As an illustration, classifying the heterogeneity of the various types of albuminurias, a quotation of the well-known textbook of Herman Strauss, entitled *Die Nephritiden*‡ may suffice. Strauss makes reference to the following types of albuminurias: "accessory, accidental, alimentary, anaphylactic, constitutional, continuous, cyclic, ephemerie, extrarenal, fugax, hematogenic, innocuous, intermittent, lordotic, minimal, neurogenic, orthostatic, periodic, persistent, physiologic, renal, renopalpatory, spurious, toxic, transitory, traumatic, tubulogen"; furthermore he speaks of albuminuria in: "anemic conditions, apoplectic insults, bacteriotoxic states, cachexias, cancer, delirium tremens, diabetes mellitus, dispositions, Graves' disease, heart affections, icteric conditions, infectious diseases, metabolic disorders, passive congestions, thoraco-compressions, traumatism (microtraumatism due to phosphaturia, oxaluria, uricaciduria)"; also during "pregnancy, parturition and puerperium; post-epileptic, premenstrual, pretuberculotic; under emotional influences; in vago-tonic conditions; after cold bath; after a general cold, after fatigue or physical strain, after marching or various sports or standing."

Under the term of "*albuminuria solaris*" we would designate transient albuminurias caused by skin reactions resulting from the exposure to sunrays. This albuminuria is *not* a febrile albuminuria. Its occurrence is not bound to the presence of fever; its precondition is a strong skin reaction only.

Under a sudden and unaccustomed exposure to sunrays, as is generally known, severe skin reaction will set in, but outside of the various degrees of burns on the skin exposed to the rays, general systemic reactions, such as chills, high temperature, general malaise, gastrointestinal symptoms, extensive herpes, etc., may follow. If albuminuria is observed under such conditions, this may range with the "*albuminuria febrilis*." Fever from any cause is followed in the overwhelmingly largest proportion of the cases by the appearance of albumin in the urine, called albuminuria febrilis, a condition,

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Jacobi's<sup>6</sup> cases it was decreased, and in Stenberg's<sup>7</sup> fourteen cases the blood cholesterol was definitely above normal. The average blood cholesterol in the six manic depressive cases reported by Gibbs<sup>8</sup> was 1627 mg, in the five depressions reported by Slight, Long and Salter<sup>9</sup> it was 239 mg, and in the manic depressive and involutional melancholia cases reported by Duncan<sup>10</sup> it was 199 mg and 216 mg, respectively. The literature on the relationship of blood cholesterol to this psychosis is thus obviously inadequate and conflicting. Further work is certainly needed to clarify this field and to stabilize the facts therein.

#### PROCEDURE

The individuals used for this work were established clinically as manic depressive. In each individual there was no demonstrable pathology other than the psychosis. This type of restriction was adhered to because there are certain pathologic states which of themselves would produce hypercholesterolemia or hypocholesterolemia, and it was felt that should individuals with pathology other than the psychosis be admitted to the group the results would not be reliable. From each individual was drawn by venipuncture, under sterile conditions, 5 cc of blood which was placed in a sterile oxalate tube, gently mixed with the oxalate, and placed in an ice box at 10° C. Tubes containing clotted blood were discarded. The blood for the controls was obtained from individuals, not patients, who were normal mentally and physically. The method used to estimate the blood cholesterol was a modified Myers Wardell<sup>11</sup>. Duplicate estimations were made on each individual and the average used in each instance.

The mental and physical activity was evaluated thus: *Mental Activity* State of cerebration, intellectual, emotional and spatial. *Increased* Irritability, quarrelsomeness, flight of ideas, distractibility, agitation, active depression, active confusion and perplexity, active delusions and hallucinations. *Decreased* Mental stupor, poverty of, or decreased cerebration. *Physical Activity* State of bodily orientation with respect to a given point in space. *Increased* Restlessness, active mannerisms, running, fighting, etc. *Decreased* Inactivity, physical stupor, physical apathy, or depression.

#### RESULTS

This study consists of 71 cases of manic depressive psychosis and 53 normal individuals. The distribution of blood cholesterol values is shown in Table I. The range of the normal group is 110 to 195 mg with a mean value of  $148 \pm 17$  mg and median value of 150 mg. There are 20 (37 per cent) cases below the normal mean and 34 (62.9 per cent) above the normal mean. The range of cholesterol values in the manic depressive group is 50 to 428 mg with a median value of 104.6 mg. The mean value is not computed due to the unbalanced distribution of blood cholesterol values. In this group there are 40 (56 per cent) cases with blood cholesterol values below normal limits, 14 (19.6 per cent) cases above and 17 (23.8 per cent) cases within normal limits. There are 49 (68.6 per cent) cases below the normal mean and 21 (29.4 per cent) above the normal mean. From these figures it is quite

color contrast involuntarily associated the idea of a possible coincidence between the sun effect and albuminuria, and she was asked to void some urine. To our surprise, the specimen showed, similarly, a definite albumin reaction, the same as her husband's.

The "experimentum crucis" of the test was then easy to produce. The couple had two children, fourteen and seventeen years of age, respectively, who were exposed to a similar sun effect, for the same length of time, and reacted with a similarly strong skin reaction. I requested a sample of urine from both of them, for immediate examination. They both contained albumin in identical amounts, in more than heavy traces. The centrifuged sediment was negative.

Thus four members of a family, being exposed under similar conditions, reacted with an equally strong skin reaction and exhibited albuminuria of a moderate, but marked, degree. Their regress and complete disappearance followed equally a uniform course. Urine specimens were received and examined daily. The maximal albumin reaction was found about thirty-six hours after the exposure. The albumin reaction of the following two days declined in intensity. After another day or two, about five or six days in all, the last albumin traces disappeared, in all the four specimens, equally.

The problem has, outside of its scientific significance, also some practical aspects. Without this knowledge, in many cases, when patients reporting for examination for any reason (to dispensary, to doctor's office, to insurance companies) during a hot July or August day, twenty-four or forty-eight hours after an exposure to a strong sun, and albumin was found in the urine, the latter would improperly be evaluated as to its classification or significance.

If, among others, H. S. Diehl and C. A. McKinley\* found in a group of 20,000 young healthy men an incidence of albuminuria in 1,065 individuals (5.32 per cent), without the knowledge of albuminuria solaris, one cannot authoritatively state that the source of the unknown or unexpected albuminuria, at least in a certain percentage of the cases, might not have been explained in an eventual sun effect. This holds true of albuminurias of other etiology, where postural, emotional or any other cyclic or transient influences were found or supposed to exist.

30 EAST NINETY-SECOND STREET

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\*Diehl, H. S., and McKinley, C. A.: Arch. Int. Med. 49: No. 1, 1932.

decreased (24 cases), the median is quite low (98.5 and 110). Where the physical activity is increased, 62 per cent of the cases have blood cholesterol values below normal and 5.4 per cent of the cases have values above. Where the physical activity is decreased, 49.9 per cent of the cases have values below normal and 29.1 per cent above.

TABLE II\*

CORRELATION OF BLOOD CHOLESTEROL AND TYPE OF MENTAL AND PHYSICAL ACTIVITY

	P I	P D	M I	M D	P I & M I	P D & M D	P I & M D	P D & M I	P N & M I	P N & M D
Median	98.5	110.0	110.0	115.0	105.0	115.0				
Per cent below N	62.1	49.9	65.7	48.5	64.3	47.3	56.8	60	75	42.6
Per cent within N	29.7	20.8	26.3	21.2	32.1	21.0	28.4	20	~	14.2
Per cent below N mean	78.3	62.4	78.9	60.6	78.5	57.8	85.2	80	75	42.6
Per cent above N	5.4	29.1	7.9	30.3	3.6	31.5	14.2	20	25	42.6
Total cases	36	24	38	33	28	19	7	5	4	7

\*Abbreviations used: P Physical activity, M mental activity, I increased, N normal, D decreased.

In those cases where the mental activity is increased (38 cases) or decreased (33 cases) and considered alone, the median is low (110 and 115). Where the mental activity is increased, 65.7 per cent of the cases have blood cholesterol values below normal and 7.9 per cent above, where the mental activity was decreased, 48.5 per cent are below normal and 30.3 per cent above.

In those cases where both the mental and physical activity are increased (29 cases) or decreased (19 cases), the median value of the blood cholesterol is low (105 and 115). Where both the mental and physical activity are increased, 64.3 per cent of the cases have blood cholesterol values below normal and 3.6 per cent above. Where both mental and physical activity are decreased 47.3 per cent of the cases have blood cholesterol values below normal and 31.5 per cent above.

There are seven cases where the mental activity is decreased and the physical activity increased. Of these, 56.8 per cent have blood cholesterol values below normal and 14.2 per cent above. There are, also, seven cases where the mental activity is decreased and physical activity normal. Of these, 42.6 per cent have blood cholesterol values below normal and 42.6 per cent above. In four cases where the mental activity is increased and the physical activity is normal, 75 per cent have blood cholesterol values below normal and 25 per cent above. And in five cases where the mental activity is increased and the physical activity decreased, 60 per cent are below normal and 20 per cent above.

Therefore, in the manic depressive psychosis characterized by increased physical or mental activity or both, there is a definite tendency to an accompanying low blood cholesterol. Where there is an increased physical state accompanied by a decreased mental state there is a definite tendency for an accompanying low blood cholesterol. Where the psychosis is characterized by a decreased physical or mental activity or both there is a less definite tendency for an accompanying low blood cholesterol, but rather a tendency

to determine if such a relationship could be established and to trace it where possible. In this group, no patient gave a history of having had cyclic vomiting or any other serious metabolic upset prior to the incidence of his diabetes; nor could I find any such case in my private practice.

In the group, the most common childhood disease outside of measles was whooping cough, which occurred in 14 of the 40 cases. Thirty-three per cent of the males and 36 per cent of the females were Jews. In 2 of the cases there was direct family history of diabetes occurring in the father, and in 6 cases in the collaterals.

Obesity, with or without evident disturbances of fat metabolism, is a commonly observed prediabetic state of the diabetic patient of middle age. In the juvenile type of diabetes, herein considered, this relationship does not exist.

#### ENDOCRINE FACTORS

The sex development in the juvenile group seems to be well balanced. Three patients were diminutive and showed impaired growth. Two of these were given anterior pituitary extract in addition to insulin therapy with very little stimulation of growth. The tendency was toward lack of growth rather than excessive growth. The relation of the pituitary gland to the metabolism of the diabetic will be considered in a future article.

#### BLOOD CHEMISTRY

Blood sugar readings in the group studied reached as high as 400 and 500 mg. per 100 c.c. It is recognized that with dietetic regimen alone, blood sugar fluctuates most markedly.<sup>11</sup> There was no relationship between the height of the blood sugar and the reactions described.

The calcium and phosphorus determinations of the group ranged from a low of 8.3 mg. per 100 c.c. of calcium and 3.5 mg. of phosphorus to a high of 12.1 calcium and 3.14 phosphorus; and from a low phosphorus of 2.3 and 9.75 calcium to a high of 4.6 phosphorus and 11.1 calcium. The diabetic calcium-phosphorus ratio showed no variation from the normal or average readings.

#### INSULIN DOSAGE

The insulin preparations used were straight beef extracts, and the mixed extracts were obtained from beef, sheep, and pig. The mixed pancreatic extracts appeared to give more satisfactory results than the straight beef extract, but the preparations used seemed to bear no relation to the reactions under consideration. The dosage varied from a minimum of 20 units a day to a maximum of 141 units, the average daily dose being  $61\frac{1}{2}$  units. Of the group exhibiting reactions, one was receiving 20 units daily, one 44 units and the others over 50 units.

The occurrence of reactions unaccompanied by hypoglycemia has been described. In this group, there was no relationship between the brand and dose of the insulin. However, the possibility of some other factor in the insulin cannot be eliminated.

In addition to these conditions which can alter the blood cholesterol, there are to be considered the endocrine glands, the autonomic nervous system, and the emotions. Although no clinical evidence of an abnormality of the first two is present, alterations in the endocrine and autonomic nervous system can exist without the presence of a measurable clinical syndrome. That the endocrine and autonomic nervous systems can alter the blood cholesterol has been shown many times both clinically and experimentally. And there is adequate evidence in this and the other papers<sup>1-10</sup> that altered emotions are accompanied in many instances by abnormalities in the blood cholesterol. Likewise it is well known that there is a close alliance between the endocrine glands, the autonomic nervous system and the emotions and that an alteration of one of these can affect profoundly the other two. It is very possible that the abnormality in the blood cholesterol in the manic depressive psychosis, is, to a certain extent, due to some imbalance of this triad. More cannot be said at this time, but further work in this field, it is hoped, will clarify the situation.

#### SUMMARY

1 A study of the blood cholesterol in the manic depressive psychosis is presented.

2 Abnormal blood cholesterol values are found in 76.2 per cent of the cases.

3 There is definite evidence that there is some correlation between the state of mental and physical activity and blood cholesterol.

#### REFERENCES

- 1 Pighini, G. Ueber die Menge Cholesterins und Oxysterins des Serums bei verschiedenen Formen von Geisteskrankheiten, *Ztschr f d ges Neurol u Psychiat* 4: 629, 1910-11.
- 2 Tsuchiya, S. Über Cholesterin in der Cerebrospinalflüssigkeit, *Ztschr f Neurol u Psychiat* 90: 255, 1924.
- 3 Targowla, R., Badonnel, M., and Berman, A. Cholesterolemia in Mental Diseases, *Encephale* 18: 138, 1923.
- 4 Parhon, C. J., and Parhon, M. Cholesteremia in Mental Affections, *Encephale* 20: 48, 1925.
- 5 Ornstein, I. Cholesteremia in Insane, *Compt rend Soc de biol* 93: 1622, 1926.
- 6 Jacobi, W. Cholesterinstoffwechsel und Konstitutionspathologie der Psychosen, *Monatsschr f Psychiat u. Neurol* 62: 296, 1926.
- 7 Stenberg, S. Psychosis and Blood Lipoids. Quantitative Variations of Total Cholesterol and Total Fatty Acids in the Blood. 1. Manic Depressive Psychosis, *Acta med Scandinav* 71: 559, 1929.
- 8 Gibbs, C. E. The Suprarenal Cortex and Blood Cholesterol in Dementia Praecox, *Am J Psychiat* 5: 189, 1925.
- 9 Shlight, D., Long, C. N. H., and Salter, R. W. Plasma Lipoids in Mental Depression, *Am J Psychiat* 13: 141, 1933.
- 10 Duncan, A. G. Serum Cholesterol in Mental Disorders, *J Ment Sc* 76: 284, 1930.
- 11 Schube, P. G. A Method of Measuring Cholesterol, *J Lab & Clin Med* 18: 306, 1932.

of patients who responded in the same way to administration of Fowler's solution, 3 minims twice daily.

In regard to dietary control, the author has always felt and impressed upon the juvenile diabetic that the diabetes is a maladjustment rather than a serious disease, and that the condition is amenable to adjustment of diet and the use of insulin. The diets were made ample and the carbohydrate-fat ratio of 3 to 1 was established.

The value of the low caloric diet is that of a stepping-stone to the work diet or permanent diet. The work diet, as the name implies, forms the basis of the active life diet of the individual.

#### REPORT OF CASES

Full details of the 40 cases studied are omitted because of space limitation. The following case reports have been selected as most typical of the group:

CASE 1.—White male, English, twenty-two years of age, 5 feet 7 inches tall, weight 154 pounds, had diabetes mellitus for four years. He was admitted to the hospital for middle ear infection, which cleared up spontaneously about two weeks after admission.

Father died at forty-two years of age of pulmonary tuberculosis, and the mother now has active tuberculosis. The only previous diseases were whooping cough, measles, and chickenpox. Physical examination of the patient was essentially negative, except that electrocardiography revealed a picture of sinus tachycardia and myocardial damage.

Ordered on a diet of: carbohydrates 60, proteins 50, fats 125, calories 1,600, with 30 units of insulin; but violated rules and ate the same as the rest of the family. A check revealed that the family ate about: carbohydrates 250, proteins 100, fats 120, calories 2,500. He was placed on this diet and 120 units of insulin, which maintained him with rapid gain in weight and strength. His blood sugar on admission was 410 mg. per 100 c.c. His fasting sugar was 213 mg. per 100 c.c.; and the blood taken two hours after breakfast, with insulin, was 150 mg. per 100 c.c.

On discharge from the hospital he complained of attacks of dizziness not relieved by administration of sugar. The pH of the urine at 11:30 A.M. was 7.8. The average for the week taken at 2 P.M. was 7.5.

The patient was placed on an acid carbohydrate diet of: carbohydrates 250, proteins 100, fats 120, calories 2,500, and has not complained of these symptoms since. When tested in the clinic, the urine pH at 11 A.M. was 7.0.

CASE 2.—White female, Irish, aged ten years, height 4 feet 7 inches, weight 75 pounds. The mother and father are living. The mother has pulmonary tuberculosis, and the father has chronic alcoholism.

The child was operated upon for a ruptured appendix in November, 1932. She has had scarlet fever, whooping cough, and measles.

In August, 1933, the patient suddenly developed polyuria, loss of weight, thirst, hunger, weakness, and finally coma. As soon as diagnosis of diabetes mellitus was made, she was placed upon a diet of: carbohydrates 200, proteins 100, fats 100, calories 2,100, and 44 units of insulin. She recovered quickly from coma.

In the early summer of 1935, the patient had six attacks of amnesia and was mentally confused every day for a period of time around the noon hour. This condition was quickly relieved by food. On July 4, 1935, she had an attack of convulsions. She suddenly began to scream and shriek while at lunch. This was followed by throwing the head to the right, general convulsions, cyanosis, frothing at the mouth, and unconsciousness, lasting forty-five minutes. This attack was not relieved by administration of sugar or any medication.

Following the attacks the urine was found to have a pH of 7.4 in the early afternoon. The patient was treated with an acid carbohydrate diet and 4 minims of Fowler's solution per day. The urine pH was found to be constantly 7.0. There were no further attacks.

characterized by a mild, transient albuminuria of the nephrosis type, persisting during the feverish period, without the appearance of red blood cells or of casts in the sediment, or without any noticeable impairment of the kidney function. Dermatitis solaris with fever may correctly be classified in this group. However, in albuminuria solaris albumin is detected in the urine also in the "afebrile" cases of the dermatitis solaris; in other words albuminuria will be noticed in cases with milder skin reactions, when general symptoms are hardly noticeable and, in particular, fever is absent. Albuminuria undoubtedly serves, under such conditions, as a more sensitive accompaniment than the other generalized symptoms. In cases of albuminuria solaris albumin is detectable in the urine as long as new large areas of the skin are involved, characterized by the classical calor, turgor, rubor, dolor, and functio lesa, even though these syndromes are not strong enough to produce any noticeable fever or any other more marked general systemic reactions.

The albuminuria lasts as a rule as long as new large skin areas are involved, usually for a few days, rarely longer than a week. As soon as the acuteness of the process fades away, the redness subsides, even prior to the peeling of the skin or brownish pigmentation, the albuminuria disappears.

After one exposure to the sun, when the course of the albuminuria can best be seen and studied, one will notice that the albuminuria reaches its height after twenty-four hours to the exposure to the sun, and will remain unchanged for another twenty-four or thirty-six hours. Two or three days after the exposure, the albumin diminishes, until in another day or two it disappears entirely.

According to the degree of the burns of the skin, the amount of the albumin may be quite noticeable, in fact, considerable, ranging around a level of a  $\frac{1}{4}$  or  $\frac{1}{2}$  pro mille, producing more than heavy traces, still with a negative finding in the centrifuged sediment. The albumin can be demonstrated with any of the usual methods (boiling of the acidified urine, the sulphosalicylic acid test, or Esbach's reaction, etc.).

The renal effect of the acute skin reaction, due to exposure to strong sunrays, is quite striking, the relative severity of the albumin reaction being out of proportion to the general systemic effect.

A few illustrative cases demonstrating the presence, frequency, uniformity and course of the albuminuria, simultaneously describing the way of its first detection, should be recorded.

A patient, A. D., fifty, white, male, who used to report for a periodic health examination twice a year, and whose urine in the past proved always to be free of albumin, happened to report for a routine office examination on one of the warmest days of July in 1935, incidentally after his first seashore sunbath in that year. Although the acuteness of the skin reaction was quite conspicuous, there was no fever or general malaise, and the skin was free of blisters. The physical examination was negative, except for a very definite albumin reaction in the urine, a finding unexpected and unexplained. The centrifuged sediment was entirely negative. Patient was ordered to bed, put on a strict diet and a specimen of urine was requested for the following day, for examination.

The specimen was brought to the office by the wife of the patient. She had a striking sunburn, the result of a similar sun effect, being in sharp contrast to her blond hair. The

patient developed periods of *petit mal*; urinary sugar was present during these attacks. They would occur at intervals of every two or three weeks. These attacks interfered with the child's attendance at school. Examination of urine at these times revealed an alkaline tide which persisted in spite of dietetic replacement. Fowler's solution was administered, 3 minims twice a day. The spells ceased after two weeks of administration. At this time the child was taken to Pittsburgh.

December, 1935, patient weighed 50 pounds and was 4 feet 1 inch tall. Mother reported that the child had had no spells as heretofore described and he was now being given the pituitary growth hormone. He was 4 feet 1 inch tall and weighed 50 pounds, while the younger brother was 4 feet 4 inches tall and weighed 60 pounds.

CASE 5.—Male physician, aged twenty-four years, developed diabetes at the age of nineteen years. It was discovered upon examination of the urine. He was 5 feet 5 inches tall and weighed 130 pounds. He had severe whooping cough when a child. His father had died of diabetes at the age of 51. Two brothers aged 26 and 27, respectively, had diabetes.

He was placed upon a diet of carbohydrates 260, proteins 80, fats 140, without insulin. Between 11 A.M. and 1 P.M. he observed reactions during which he would perspire freely and tremble. There was inability to concentrate. This was not associated with hunger.

During one attack, the blood contained 200 mg. of dextrose per 100 c.c. Urine examined at these times showed marked alkaline reaction ranging from pH 7.0 to pH 7.5. Diet was changed and acid-producing fruit substituted for the alkaline-producing, with complete alleviation of these symptoms and a change in the urinary reaction from alkaline to neutral and acid.

#### SUMMARY

In a series of 40 cases of juvenile diabetes mellitus, insulin-like reactions, unrelated to hypoglycemia, were observed in 16. In many cases the reactions resembled attacks of *petit* or *grand mal*. Of uniform occurrence in these cases was a definite alkaline tide, the alkaline pH of the urine reaching its peak about 2 P.M. An acid-producing diet proved remarkably effective both in bringing the reaction of the urine within the acid range and relieving the insulin-like reactions of epileptiform seizures. Administration of small doses of Fowler's solution often had a similar effect.

#### REFERENCES

1. Edsall, D. L.: A Preliminary Communication Concerning the Nature and Treatment of Recurrent Vomiting in Children, *Am. J. M. Sc.* 125: 629, 1903.
2. Hecker, P.: Periodisches Erbrechen mit Acetonämie, *Ergebn. d. inn. Med. u. Kinderh.* 7: 242, 1911.
3. Marfan: Diagnostic et traitement des vomissements périodiques avec acétonémie, *Bull. méd.* 30: 605, 1916.
4. Marriott, W. McK., and Howland, J.: Phosphate Retention as a Factor in the Production of Nephritis, *Arch. Int. Med.* 18: 708, 1916.
5. Henderson, L. J., and Palmer, W. W.: On the Several Factors of Acid Excretion in Nephritis, *J. Biol. Chem.* 21: 37, 1915.
6. Palmer, W. W., and Henderson, L. J.: Clinical Studies on Acid Base Equilibrium and the Nature of Acidosis, *Arch. Int. Med.* 12: 153, 1913.
7. Revillet: Coma chez une diabétique sans acétonurie, *Lyon méd.* 122: 815, 1914.
8. Rosenbloom, J.: A Form of Diabetic Coma Not Due to the Acetone Bodies, *New York M. J.* 102: 294, 1915.
9. McCaskey, G. W.: A Case of Fatal Diabetic Coma Without Diacetic or Betaoxybutyric Acid, *J. A. M. A.* 66: 350, 1916.
10. Starr, P., and Fitz, R.: The Excretion of Organic Acids in the Urine of Patients With Diabetes Mellitus, *Arch. Int. Med.* 33: 97, 1924.
11. Feinblatt, H. M.: Hyperglycemia—Based Upon a Study of 2,000 Blood Chemical Analyses, *J. LAB. & CLIN. MED.* 8: 500, 1923.
12. Feinblatt, H. M.: Three Cases of Endocrine Disease With Pronounced Asthenia, *M. Clin. North America* 11: 107, 1928.
13. Feinblatt, H. M., and Sherman, I.: Report of a Very Severe Case of Juvenile Diabetic Coma in Which Combined Treatment With Insulin and Blood Transfusion Resulted in Prompt Recovery, *J. LAB. & CLIN. MED.* 11: 63, 1925.

## JUVENILE DIABETES MELLITUS\*

### A STUDY OF INSULIN-LIKE REACTIONS UNRELATED TO HYPOGLYCEMIA

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**D**URING observations on the juvenile type of diabetes mellitus over a period of fifteen years, the senior author has been impressed with the frequent incidence of a group of insulin-like reactions unassociated with hunger and unrelated to the amount of sugar or ketones in the urine or to the level of sugar in the blood.

These reactions have varied from a momentary lapse of consciousness to epileptiform convulsions resembling grand mal. It was found through routine examinations of the urine that they occurred simultaneously with an alkaline tide in the urine, and that they could be relieved readily by an acid-producing diet.

#### LITERATURE

The frequency of ketonuric acidosis in diabetes is too well recognized to be given special consideration here. The incidence of severe ketonuric acidosis in the nondiabetic has been especially well recognized in this country. This was described by Edsall<sup>1</sup> in 1903, Hecker<sup>2</sup> in 1911, and Marfan<sup>3</sup> in 1916 as cyclic vomiting and a disease of childhood. The severity and acuteness of onset and the rapidity of its subsidence are its outstanding characteristics. The infrequency of coma and the contrast of symptoms with those of diabetic ketonuria indicate a basic difference in the character of the acidosis. The literature is woefully lacking in pertinent pathologic data concerning cyclic vomiting. The authors' single postmortem observation showed extensive fatty degeneration of the liver with almost complete replacement of liver cells. There were no other findings.

It has been assumed that the acidosis of nephritis is due to the failure of the kidneys to excrete the acid products of normal metabolism, causing phosphoric acid retention. Marriott and Howland<sup>4</sup> in 1916 found a marked increase in the organic (acid) phosphate of the serum in nephritic patients with acidosis. Henderson and Palmer<sup>5, 6</sup> in 1915 found the ammonia (titratable acid) abnormally high. The incidence of nonketogenic acidosis in the diabetic may be aggravated by the underlying progressive degenerative lesions in the kidney tubule cells. The severity of this type of acidosis may be extreme, even fatal.<sup>7-10</sup>

#### HISTORY

In a careful review of the natural history of the juvenile diabetic, it was hoped that some relationship might be established between metabolic disturbances in earlier life and the onset of the diabetes. Because of the frequency of cyclic vomiting in diseases of children, detailed historic references were made

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This report is based on study of a group of forty cases of juvenile diabetes selected from the out-patient clinic at the Kings County Hospital. The patients were seen at regular intervals, at which time their weekly supply of insulin was replenished gratuitously.

That amyloid deposits are found in the heart was known to Virchow.<sup>1</sup> From time to time cases are recorded, in which the heart is involved as part of a generalized process. Rare, however, are the instances in which myocardial amyloidosis ranks as the primary cause of death. In 1886, Wild<sup>11</sup> reported a case in which the patient, who suffered from erysipelas, presented the clinical picture of cardiac failure. At autopsy extensive deposits of amyloid were found throughout the heart. In the case of Steinhäus,<sup>12</sup> marked infiltration of the heart and gastrointestinal tract accounted for the symptoms of heart failure and intestinal upset. In 1926, Silwer and Lindblom<sup>13</sup> presented the case of a male of thirty-four years with symptoms of cardiac insufficiency for ten months. He showed marked signs of cardiac failure, and apparently died from this cause. Autopsy revealed amyloidosis of heart, liver, and spleen, but no etiologic factor could be demonstrated.

In Larsen's recent example,<sup>14</sup> the patient had symptoms of left-sided heart failure for about a year. In this case the amyloid deposits were located chiefly in heart, lungs, and kidneys, the liver and spleen being uninvolved. Perla and Gross<sup>8</sup> have described three cases of atypical amyloid disease, in one of which death was due to widespread replacement of cardiac muscle by amyloid. Reimann, Koucky and Eklund<sup>15</sup> recently studied amyloidosis in a patient who died of a terminal peritonitis. The organs containing amyloid were tongue, heart, lungs, esophagus, and pelvic organs.

Though the two cases, the details of which follow, possess certain clinical features in common, the differences in the pathologic findings are striking.

**CASE 1.\*—Clinical History.**—The patient, a white female of fifty-one years, was admitted on March 13, 1927, to the Medical Service of the Toronto General Hospital under Professor Duncan Graham. She complained of feeling "run down" for about the past seven years with only occasional short periods of good health. For a year she had felt weaker, had lost 20 pounds in weight and suffered from an intermittent dry cough. These symptoms became progressively worse, particularly in the three weeks prior to admission, most of which time was spent in bed. She confessed to slight shortness of breath on exertion but denied any swelling of the feet, palpitation or precordial pain. Except for a moderate degree of constipation for many years, functional inquiry revealed no other complaints. No significant facts were elicited in the past history.

**Physical Examination.**—In appearance the patient was rather pale and undernourished. The heart was moderately enlarged to percussion; no murmurs were heard. The blood pressure was 76/48. There was no edema of the dependent parts. Examination of the chest revealed impairment of resonance and breath sounds at both lung bases; a few râles were heard in the left axilla. There were no other findings of importance.

**Laboratory Record.**—The urine had a specific gravity of 1.017, showed a faint trace of albumin and contained a few pus cells.

The hemoglobin was 88 per cent (Sahli); the red cells numbered 5 million per c.mm. and the white cells, 6,700. The blood smear was not remarkable and the differential white count was normal.

The blood Wassermann and Kahn tests were negative.

The electrocardiogram showed left ventricular preponderance and low voltage.

Roentgenograms of chest, gastrointestinal tract, gallbladder, and vertebral column were negative.

**Clinical Diagnosis.**—Chronic degenerative myocarditis; cardiac hypertrophy; myocardial insufficiency.

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\*This case is reported through the court autopsy.

## REACTIONS UNRELATED TO HYPOGLYCEMIA

The occurrence of reactions unaccompanied by hypoglycemia in the case of a woman aged fifty nine years has already been described by the senior author.<sup>17</sup> Although insulin dosage ranged from 190 units to 400 units daily, there was persistent hyperglycemia with ketonuria and glycosuria. In this case, the insulin failed to bring the blood sugar down to a normal level at any time, yet there occurred reactions, at times accompanied by generalized twitchings and loss of consciousness, lasting as long as an hour. Obviously, the dosage of insulin was not an essential factor in the causation of these attacks.

Of the group reported, 16 cases demonstrated the reactions described, which ranged from a momentary lapse of consciousness to epileptiform convulsions. These reactions were not relieved by administration of sugar and were not associated with hypoglycemia nor were they associated with skin manifestations, which would ordinarily occur in the event of foreign protein reactions.

## PH OF URINE

Determination of the hydrogen ion concentration of the blood was considered unreliable and impractical in this group of cases. A simple method of determining the pH of the urine by the use of an indicator solution was adopted. This was made up and supplied to the individual patient, who was instructed as to its use. The following formula was used:

Methyl red	20 mg
Bromthymol blue	40 mg
Ethyl alcohol	50 cc
Add	
N/5 NaOH	2 cc
Distilled water	150 cc
Five per cent phenol	20 cc

*Technic*—Float 6 drops of the test solution on half a test tube of urine. Color ring forming will be the same color as the urine for a neutral reaction, red for acid and green for alkaline reaction.

pH 7.5	Dark green
pH 7.0	Green
pH 6.5	Same color as specimen
pH 6.0	Red
pH 5.5	Dark red

Urines were tested several times during the day, but the highest point of alkalinity in these cases was reached in urines examined about 2 p.m.

## TREATMENT

It was found that the alkaline tide in this group could be balanced by an acid producing diet and that such a dietary correction resulted in relief of symptoms and reactions and simultaneous shift of the urinary reaction to the acid side. This dietetic treatment proved quite as effective as use of the ketogenic diet for idiopathic epilepsy in children. There were a few exceptions in cases

edema, she began to have shortness of breath on exertion which grew progressively worse. For two months her abdomen had been slowly increasing in size. No facts of significance were revealed in the functional inquiry or the past history.

*Physical Examination*—The patient appeared quite ill, dyspnea being a prominent feature. Pitting edema was present over the whole body up to the level of the third ribs anteriorly. The legs were swollen and tense. The veins of the neck were engorged and pulsating.

The borders of the heart could not be percussed due to edema and tenderness of the chest wall. The heart rate averaged 100 per minute, the rhythm being normal. The blood pressure was 130/90. The abdomen contained free fluid.

*Laboratory Record*—The urine had a specific gravity of 1.026 and showed albumin (3+) with an occasional hyaline cast and pus cell.

The blood smear and differential white count were not abnormal and the hemoglobin was 77 per cent (Sahlb). The blood nonprotein nitrogen was 44 mg.

Unfortunately a blood Wassermann was not obtained.

The electrocardiogram revealed right axis deviation with a voltage = 2 mm.

*Clinical Diagnosis*—Chronic degenerative myocarditis, myocardial insufficiency.

The day following admission, the patient's dyspnea grew worse, and the right chest was aspirated, 900 c.c. of clear, yellow, sterile fluid being removed. Later the same day, she developed acute pulmonary edema, successfully treated by atropine and venesection. The next day she rapidly became worse and died.

*Autopsy Findings*—The legs were swollen and tense, and pitting edema was present over the body up to the level of the manubrium sterni. The right pleural cavity contained 600 c.c. of clear yellow fluid, and the left, 800 c.c. Both lung bases showed hypostatic pneumonia. About 600 c.c. of yellow fluid was obtained from the peritoneal cavity. The liver and kidneys were not remarkable in the gross. With the exception of the spleen, none of the abdominal organs gave a positive reaction with iodine.

No lesion could be found to form a logical basis for the development of amyloidosis.

*Heart*—The heart was slightly enlarged, measuring 12 by 10 by 6 cm., and was definitely heavier than normal, weighing 360 gm. The pericardium was smooth except for a small "milk spot" on the anterior surface. The myocardium felt firm and waxy and the cut surface was a pale, homogeneous, glassy, reddish brown color. Several portions were soaked in iodine and almost immediately the tissue became flecked with dark brown streaks and dots. The endocardium and valve cusps were smooth and glistening throughout.

*Spleen*—The spleen was large and heavy, measuring 11 by 7.5 by 5 cm. and weighing 270 gm. It was extremely firm in consistency, like hard rubber, and little impression could be made on it with the fingers. The cut surface was quite hard, bright rose red in color, and the normal markings were obliterated. On treating with iodine, the surface became a deep mahogany brown. The distribution was unusual, the staining being diffuse throughout. Attached to the hilus of the spleen were two small accessory spleens, the larger measuring 2.5 cm. in diameter. Their cut surfaces were similar in appearance and in consistency to the larger organ. Both turned a dark brown on the application of iodine.

None of the other organs presented any important gross abnormality and none reacted positively to iodine.

*Microscopic Examination*—*Heart*—Sections taken from all portions of the heart showed extensive infiltration and destruction of muscle by amyloid. For the most part the lesions were diffuse in character. Between the individual muscle cells lay thin strands of pale fibrillar material which was stained a faint pink by Congo red. The small nodular masses so common in the previous case were seen infrequently. They were located chiefly in the fibrous trabeculae, and it is quite possible as Larsen<sup>14</sup> suggests, that these were all primarily derived from a blood vessel. The large patches which were not numerous appeared to have resulted from the disappearance of muscle and the coalescence of many small strands.

With respect to distribution, no part of the myocardium was free from the lesions. The ventricular muscle appeared to be affected to a greater degree than the auricular. The

The physical examination revealed atrophy of the muscles of both thighs and arms. This occurred at the site of injection of the insulin. The rest of the findings were essentially negative.

CASE 3—(Previously reported<sup>13</sup>) H. M., Jewish boy, developed diabetes in the summer of 1923, when he was nine years of age. There had been a gradual loss of strength and weight and pain in the epigastrium. He was treated with insulin and diet and urine became sugar free.

Unfortunately, he fell into the hands of a quack, upon whose advice insulin was stopped on Sept. 16, 1924, and a liberal diet was instituted. On September 18, patient was admitted to the hospital in a comatose state. He was given insulin, clyses, and blood transfusions. Improvement was progressive and rapid, and he was discharged from the hospital on September 23.

Dietetic regimen, on account of his low weight, was gradually changed. At the end of six months he was getting 2,000 calories: carbohydrates 200, proteins 85, and fats 100. Insulin was increased from 30 units daily to 22 units in the morning and 24 at night. Patient was perfectly well except for occasional insulin reactions, although he had never been entirely free from glycosuria. Because each attempt to increase the insulin content resulted in insulin shock, patient was permitted to run glycosuria, once or twice daily, but there was at least one clear specimen during the day.

He noticed while at college during the school year of 1931 that he was subject to temporary periods when there was a loss of memory. At these times his school notes were incomplete. The state of unconsciousness was momentary and lasted at the most from one to four seconds. The lapses occurred in the afternoon between one and three o'clock. Urinary examinations made from one to three hours after these spells were positive for sugar, usually showing a trace. Blood sugar determinations were made repeatedly at this time of the day and on no occasion revealed hypoglycemia.

On May 5, 1932, the character of the reactions changed and the patient developed definite epilepsy. Examinations of the urine immediately after this occurred were positive for sugar but alkaline in reaction. A second epileptic attack occurred on May 10, at which time urine examination also revealed presence of sugar and an alkaline reaction. Alkalinity was persistent for a number of days after this.

July 7, patient again had epileptic attack. Examination of urine showed sugar present, alkaline reaction. Van Slyke  $\text{CO}_2$  was 76. August 15, patient had an epileptic attack. September 17, epileptic seizure. October 22, there was another epileptic attack. During intervals in this period, patient again noticed the short lapses of memory. December 1, epileptic seizure. Patient was placed upon an acid producing diet and was given Fowler's solution. The insulin dosage was maintained and the diet was unchanged except for substitution of acid juices.

He has been seen at intervals for the past three years and has had no complaints. There have been no repetitions of epileptic attacks.

CASE 4—D. B. was first observed in November, 1925. He was then two years and three months. He was 3 feet 2 inches tall and weighed 30 pounds. His family history was negative for diabetes. He was emaciated and complained of thirst and frequency of urination. Two months prior to illness he had had an attack of tonsillitis and during the three weeks prior to observation showed evidence of marked thirst and polyuria. Urine sugar was 3 per cent and blood sugar was 240 mg per 100 c.c. Ketonuria was present.

He was placed upon a diet of carbohydrates 80, proteins 50, fats 55, and given 6 units of insulin morning and night. The diet was gradually increased and the insulin dosage was maintained.

October, 1927, the patient had enlargement of the liver. He felt well and was active. He had exhibited insulin shock reactions with this small dosage of insulin. April, 1930, weight was 40 pounds, height was 3 feet 3 inches. There was an increase of only  $1\frac{1}{4}$  inches in five years. At this time, his twin brother developed pneumonia and died three hours after onset.

In 1931, insulin dosage was increased to 8 units morning and night. His brother, only four and one half years old, was taller and stronger. It was during this interval that the

Then, too, the characters of the heart lesions differed. In the one the pericardial and endocardial surfaces were studded with nodules of amyloid; in the other these surfaces were grossly uninvolved. The myocardial lesions were both nodular and diffuse in the first, but in the second, diffuse only. Whether any significance can be attached to this difference is uncertain. Is this type of nodular deposit, as Lubarsch<sup>17</sup> suggests, frequently present in atypical amyloidosis? Is there any relationship between their absence in the second case and the involvement of spleen and liver? These questions can only be answered by further research on the mode of amyloid formation.

Larsen<sup>14</sup> has described in detail the formation and spread of cardiac deposits. He believes that they always arise from a pericapillary nidus and extend into the interstitial tissue, surrounding the muscle fibers and cutting off nutrition. Occasionally invagination and penetration of the individual fibers occurs. In both of our cases the muscle cells showed marked fatty degeneration which was probably due to failing nutrition and represented a stage in the destruction of the cell. To what degree death was due to replacement by amyloid and to what degree it was due to the fatty degeneration of the remaining muscle, it is not possible to say. In any case, amyloidosis was the basic factor.

The classification of myocardial amyloidosis has been attempted by a number of authors. The simplest method is to divide the cases into two groups as does Larsen:<sup>14</sup> those in which the heart is affected as part of a widespread process, and those in which it is involved usually to the exclusion of the commonly affected other organs. Still a third class is recognized by Budd,<sup>16</sup> those in which there is generalized disease of the muscle systems. It is questionable whether such classifications serve a useful purpose other than to bring to mind the variety of distribution found. While the first case would fall into the former class, the second combines the features of both classes and bridges the gap between the two.

With respect to etiology these cases present nothing that is new. Susman<sup>19</sup> suggests that, of the substances which produce amyloidosis experimentally, the common factor is toxicity or the ability to damage tissues, and that the spleen in some way is necessary to the process although the formation is of local manufacture. This is not wholly inconsistent with the results of Smetana's<sup>18, 20</sup> experiments which demonstrated the important rôle of the reticulo-endothelial system in amyloid formation. In Warren's<sup>21</sup> example of amyloidosis of the muscles, he attributed it to "a widespread perversion of fibroblastic function." The findings in the above cases do nothing to refute either contention.

#### SUMMARY

1. Two cases of idiopathic myocardial amyloidosis are described.
2. The various pathologic features are discussed and compared with previously reported cases.

#### REFERENCES

1. Virchow, R.: *Cellular Pathology* (translated by F. Chance), American ed. 7, New York, 1858, Robert M. De Witt, p. 409.
2. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, 1925, W. B. Saunders Co., pp. 469-475.

## IDIOPATHIC AMYLOID DISEASE OF THE HEART\*

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THOUGH "waxy" or "lardaceous" disease was recognized before Virchow's time, these terms referred to a variety of ill defined conditions many of which would probably not be so classified today. He, it was, who in 1853 discovered that organs containing the substance which he named "amyloid," possessed a characteristic staining reaction with iodine and sulphuric acid.<sup>1</sup> Since that time numerous studies and case reports on amyloidosis have appeared in the literature, and the subject has been attacked from many different angles. In spite of these investigations, the exact nature of amyloid is still a matter of dispute,<sup>2</sup> but it seems certain that its composition varies in different cases and even in different organs.

Most observers agree that amyloidosis is now much less common than in the past.<sup>3</sup> This decrease they attribute to improvement in the treatment of the chronic suppurative diseases which, in some fashion still undetermined, frequently form the basis for its development. Some of this decrease is counteracted by the increased frequency of the clinical diagnosis made possible by the introduction of Bennhold's Congo red test. Not only has the diagnosis in doubtful cases been confirmed by the use of this test, but also it has been found that spontaneous regression may occur, as in the cases of Walker<sup>4</sup> and Reimann.<sup>5</sup>

In many instances, however, a suppurative focus is lacking, and the fault must be laid at the door of the associated disease, such as carcinoma, syphilis, leucemia, or myeloma. Though the presence of one or other of these conditions may afford an easy but unsatisfactory explanation of the etiology, occasionally no such background can be discovered. Idiopathic amyloidosis was known as early as 1856 when Wilks<sup>6</sup> reported several cases. Its infrequent appearance has nearly always been the occasion for interesting but fruitless speculation as to the cause.

Practically every organ in the body has been the site of amyloid deposits, though liver, spleen, and kidney are by far the commonest locations. In spite of the often widespread distribution of the process and the involvement of important organs amyloidosis is uncommonly a cause of death per se. Usually the underlying disease is responsible for the fatal termination. In rare instances though an organ may be so extensively infiltrated that the failure of a vital function results. Most frequently this occurs in the kidneys causing renal insufficiency.<sup>7, 8</sup> There have also been reported a few cases<sup>9, 10</sup> in which the adrenal tissue was almost wholly replaced by amyloid, the patients dying with the symptoms of Addison's disease.

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4 feet long so that the amount of exercise was limited. It must be pointed out here that the standard meal was not sufficient to keep a large dog well nourished, so from time to time substantial supplementary feedings were given. Care was taken, however, as to the time these feedings were given, as Ivy and Fauley<sup>2</sup> have shown that the state of hunger affects the gastric emptying time. Every effort was made to keep all experimental conditions as uniform as possible.

*Results.*—The accompanying table gives the results obtained on a series of 50 dogs. A total of 540 tests was made, including the original 200 tests published in the preliminary report.

		EMPTYING TIME OF THE STOMACH (IN HOURS)			
		AVERAGE	MEAN	LONGEST	SHORTEST
A	25 dogs	6.59	6.54	8.07	5.42
	200 tests	6.61	6.62		
B	50 dogs	6.47	6.49	8.20	5.41
	540 tests	6.54	6.52		

A = Those reported in the preliminary report.

B = Those previously reported plus 25 additional dogs.

*Discussion.*—It is distinctly interesting to note how remarkably well the new figures check with those given in the preliminary report. This very fact leads one to attach considerable significance to the values obtained. For example, the mean emptying time of the series of 25 dogs first reported was 6.54 hours, and the mean for the entire series was 6.49 hours, a difference of only 0.05 hours. It is also interesting to note that the extremes, too, seem to be well defined at about 5.41 and 8.20 hours, respectively.

We feel that the standard meal used has particular advantages, as it contained carbohydrate, protein, and fat. The milk gave it a desirable consistency, and the dogs ate it with relish, in spite of the fact that it contained considerable barium sulphate.

The dogs used were of various breeds and sizes so that the figures reported represent a good cross-section of what one might find in studying the gastric emptying time in the average laboratory animal.

*Summary.*—The emptying time of the stomach was determined in a series of 50 dogs. A total of 540 tests was made. The average emptying time of the stomach for the dogs was 6.47 hours and the average for the tests was 6.54 hours. The variations were between 5.41 and 8.20 hours, respectively. The emptying time of the stomach of each individual dog remained strikingly uniform from day to day. It is felt that these figures will be of interest and of aid to other workers.

#### REFERENCES

1. Van Liere, E. J., and Crisler, G.: Normal Emptying Time of Stomach of Dog, *Proc. Soc. Exper. Biol. & Med.* 31: 85, 1933.
2. Ivy, A. C., and Fauley, G. B.: Effect of Hunger on Emptying Time of Stomach, *Am. J. Physiol.* 91: 206, 1929.

Though the patient showed no obvious change in her condition following admission, she died suddenly on the third day

*Autopsy Findings*—Slight pitting edema was demonstrable in the lower portions of the legs. Examination of the thorax showed both visceral and parietal layers of the pericardium to be studded with firm, translucent, grayish nodules, measuring up to 1 mm in diameter. Similar deposits were likewise present on the pleura, some of these were large and plaque like, measuring up to 0.5 cm across. A few of these nodules were also found throughout the middle lobe of the right lung. The peritoneum was everywhere smooth and glistening. The mucosal surfaces of stomach, small and large intestine, and bladder, showed a diffuse scattering of small, gelatinous nodules similar to those seen on pericardium and pleura. The liver, spleen, and kidneys presented no gross evidence of amyloid disease.

Nowhere could any suppuration or other lesion be found to account for the amyloidosis.

*Heart* There was marked enlargement affecting all chambers, the heart measuring 14 by 11.8 by 7 cm. The entire pericardial surface was studded with the readily palpable, grayish nodules described above. The myocardium was firm and waxy in consistency and offered increased resistance to the knife. The wall of the left ventricle measured up to 1.3 cm in thickness, and that of the right up to 0.5 cm. The muscle was reddish brown in color and the cut surface was everywhere occupied by minute, glassy, gray areas many of which protruded slightly above the surface. Several portions of muscle were dipped in iodine. In a few moments these areas became mahogany brown in color, giving the surface a stippled appearance. Beneath the endocardium of all chambers were many fine shotty nodules. Similar deposits were present along the free margins and over the auricular surfaces of the tricuspid valve. Those on the mitral valve were fewer in number but distributed in like fashion. On the pulmonary valve these were wholly confined to the ventricular surface and on the aortic valve to the free margins. The great vessels presented no gross evidence of amyloid change.

*Microscopic Examination—Heart* There was extensive replacement of myocardium throughout by amyloid deposits which formed roughly about 60 per cent of the total area of heart muscle as seen in the sections. The distribution was both patchy and diffuse in character. Many of the patches were extremely large, some of them occupying several low power fields. In none of these were muscle fibers completely lacking, all of them containing a few, more or less isolated strands. They were quite irregular in shape and none was definitely delimited. All shaded off gradually into the less affected zones. No areas were uninvolved. Thin bands of amyloid penetrated between the individual muscle fibers. Many of the masses were small, rounded, and more or less sharply delimited. Some contained in their centers small venules which seemed narrowed and distorted, others showed no traces of blood vessels. All, however, formed an expanding mass which pushed aside and compressed the nearby muscle cells. The nodules on both the pericardium and endocardium were made up of irregularly lamellated masses of amyloid covered on their free surfaces by endothelium. The deposits on the valves were similar in appearance.

Sections stained with Sudan III showed a scattering of fine fat droplets distributed diffusely.

*Lungs* The deposits of amyloid were found in the alveolar walls which were so greatly thickened that in some places groups of the alveoli were collapsed. The nodules on the pleura were similar to those on the pericardium. The peribronchial lymph nodes were almost completely replaced by dense, homogeneous amyloid containing a few collections of anthracotic pigment.

*Gastrointestinal Tract* Everywhere the submucosa was occupied by small, irregular masses of amyloid, some of which surrounded and almost occluded the blood vessels.

No amyloid was present in liver, spleen, kidneys, or any other organs.

*CASE 2—Clinical History*—The patient, a woman fifty-nine years old, came under the care of Professor Duncan Graham at the Toronto General Hospital on Oct. 1, 1934. She had been well until four months previously, when she noticed that an unusual amount of walking caused slight swelling of her feet. At first this was present only late in the day, and was relieved by a night's rest. In the three weeks before admission, the swelling was present constantly, and it gradually increased in extent. About the same time as the onset of the

isms. This difficulty was overcome, however, by the use of washed organisms suspended in physiologic saline solution.

Cultures were grown either in liquid, or on solid media for the usual period of time. The organisms were then removed from the media by washing with sterile physiologic saline solution, in the case of the solid media, and centrifuged, or by centrifugation if liquid media were employed. They were then resuspended in sterile physiologic saline solution, aseptic technic being observed in all operations. Unless otherwise indicated, this technic was employed throughout the tests described below.

#### BACTERICIDAL ACTIVITY OF DIOTHANE SOLUTIONS

To 5 c.c. quantities of diothane solution of 1 per cent concentration was added such an amount of suspended organisms, treated as described above, as would be equivalent to 0.5 c.c. of a twenty-four-hour broth culture. After various intervals indicated in the table, transfers were made to F.D.A. formula nutrient broth. Readings were made after seven days' incubation at 37.5° C.

TABLE I  
BACTERICIDAL ACTIVITY OF DIOTHANE SOLUTION

ORGANISM	TIME OF EXPOSURE IN MINUTES						
	5	10	15	30	60	120	240
<i>B. coli</i>	-	-	-	-	-	-	-
<i>B. typhosus</i>	-	-	-	-	-	-	-
<i>Sarcina lutea</i>	-	-	-	-	-	-	-
<i>Staph. aureus</i>	+	+	+	-	-	-	-
	TIME OF EXPOSURE IN HOURS						
	1	2	4	8	24	48	96
<i>B. subtilis</i>	+	+	+	+	+	+	+

It will be noted that with the exception of *Staph. aureus* and *B. subtilis*, all of the organisms, comprising a representative group such as is usually recommended for testing the efficacy of bactericidal substances, were killed within five minutes or less. In the case of *Staph. aureus*, killing was accomplished only after exposure for thirty minutes or longer. In control tests, it had been shown previously that the strain of *Staph. aureus* used was such as to resist the action of a 1:60 phenol solution for a period of five minutes.

Preliminary tests indicated that diothane solution killed *B. subtilis*, however, microscopic examination of the cultures employed proved them to be free of spores. When older cultures were used killing was not accomplished even after exposure of four days or longer to 1 per cent diothane solution.

Assuming that there might be a difference in the killing power of diothane solution against spores in the wet state as compared with similar cultures which were dried, small amounts of washed *B. subtilis* organisms were soaked into small sterile squares of filter paper. These were then dried at incubator temperature for ninety-six hours in sterile Petri dishes. Several pieces of the paper, containing the organisms in dried form, were introduced into the 1 per cent

interventricular septum showed the most extensive involvement of all. The papillary muscles were similarly infiltrated but with the exception of a few small patches in the aortic valve, none of the valves contained amyloid. Neither pericardium nor endocardium possessed the nodules so prominent in Case 1. The few deposits seen were flattened and related to contiguous masses in the underlying myocardium.

In Sudan preparations extensive fatty degeneration of muscle was noted, many of the fibers being almost obscured by myriads of tiny fat droplets. This condition was much more marked here than in the previous case.

The larger coronary vessels were uninvolved save for a few small areas in the adventitia. Some of the small arterioles showed a mild degree of sclerosis.

*Aorta* No amyloid deposits were found.

*Liver* There was marked alteration of structure due to extreme chronic passive congestion. Many of the liver cells had disappeared and the remainder were peppered with fine droplets of fat. The amyloid deposits were located chiefly in the outer portions of the walls of the large vessels. A number of small masses were seen in the portal areas with a few thin strands passing toward the central veins between the liver cords.

*Spleen* The splenic pulp was diffusely infiltrated with amyloid. Many of the cells were completely destroyed and the rest were represented by pyknotic nuclei. Most of the malpighian corpuscles had disappeared and the remainder showed up as small collections of lymphocytes, each surrounding a compressed, distorted vessel. The accessory spleens presented the same picture.

*Kidneys* A few patches of amyloid were seen in the walls of the larger vessels.

*Adrenals* Both glands showed heavy amyloid deposits chiefly in the medulla and inner layers of the cortex.

None of the remaining organs revealed any traces of amyloid.

#### DISCUSSION

These two cases possess a number of features in common. Both occurred in middle aged women whose past history was free from any chronic illnesses. In both, the symptoms suggested cardiac failure presumably from sclerotic heart disease, and the clinical and laboratory findings supported this diagnosis. Both apparently died of heart failure. In neither could be found any adequate explanation for the development of amyloidosis. The hearts were enlarged, though unfortunately a strict comparison cannot be made as there is no record of the weight in the first case. Larsen<sup>14</sup> stated that all cases reported previous to his had had atrophic hearts. Since then, Budd,<sup>15</sup> and Peila and Gross<sup>8</sup> have recorded cases in which the heart was hypertrophied.

Considerable difficulty was experienced in obtaining characteristic staining reactions in paraffin sections of the heart. With Congo red the amyloid stained in a pale and patchy fashion. Much better results were seen in sections of spleen and liver in the second example. The fact that in atypical amyloidosis there may be failure of the usual reactions was emphasized by Lubarsch.<sup>17</sup> Another possibility is suggested by the experiments of Smetana,<sup>18</sup> who found that recently formed amyloid showed up only after intravenous injection of Congo red.

A number of important variations were also noted. The distribution of the lesions outside of the heart was quite different. In the first case these were found in lungs, pleura, and submucosa of gastrointestinal tract and bladder, in the second, the spleen, liver, and adrenals were affected.

ated lethal, or merely inhibitory action. The following Tables III and IV present the results in condensed form.

In the tables, the minus sign (-) indicates death of the organisms, the plus sign (+) indicates growth, while the letter "i" indicates that the organisms are inhibited only. It will be noted that the diothane solution is most active in the acid media and in all instances was less active in those media possessing the more alkaline reaction. It will also be seen that in the acid media all of the organisms with the exception of *B. subtilis* were killed when such media contained as low a concentration of diothane as 1 part in 400 parts of broth. *C. diphtheriae*, *B. typhosus*, and *Sarcina lutea* were readily killed, while *B. subtilis* was not

TABLE III  
BACTERIOSTATIC ACTIVITY OF DIOTHANE IN BROTH OF pH 6.8

CONCENTRATION OF DIOTHANE IN BROTH OF ORIGINAL pH OF 6.8	pH	B. TY- PHOSUS	B. COLI	STAPH. AUREUS	B. SUB- TILIS	S. LUTEA	C. DIPH- THERIAE	YEAST	PENICIL- LIUM
1: 200	5.6	-	-	-	i	-	-	-	-
1: 400	5.9	-	-	-	i	-	-	-	-
1: 800	6.4	-	+	i	i	-	-	-	-
1: 1,600	6.4	-	+	i	i	-	-	-	+
1: 3,200	6.4	-	+	+	i	-	-	+	+
1: 6,400	6.5	+	+	+	+	-	-	+	+
1:12,800	6.8	+	+	+	+	+	+	+	+

TABLE IV  
BACTERIOSTATIC ACTIVITY OF DIOTHANE IN BROTH OF pH 7.8

CONCENTRATION OF DIOTHANE IN BROTH OF ORIGINAL pH OF 7.8	pH	B. TY- PHOSUS	B. COLI	STAPH. AUREUS	B. SUB- TILIS	S. LUTEA	C. DIPH- THERIAE	YEAST	PENICIL- LIUM
1: 200	5.6	-	-	-	i	-	-	-	-
1: 400	6.6	-	+	-	+	-	-	-	-
1: 800	7.4	+	+	+	+	-	-	-	-
1: 1,600	7.6	+	+	+	+	-	-	+	+
1: 3,200	7.6	+	+	+	+	+	+	+	+
1: 6,400	7.8	+	+	+	+	+	+	+	+
1:12,800	7.8	+	+	+	+	+	+	+	+

killed in broth containing diothane to the extent of 1:200. It was significant that the growth of *B. subtilis* was readily inhibited in broth containing as little as 1 part of diothane to 3,200 parts of broth. It was found further that broth containing varying amounts of diothane is lethal and strongly inhibitory to the strains of yeast and molds employed in the tests.

The change in reaction in the media following the addition of diothane is noted by the pH values recorded in the second column.

#### CONCLUSIONS

It must be concluded that while a 1 per cent diothane solution is rather strongly bactericidal against most of the organisms usually employed to measure such activity, since it does not kill *B. subtilis* spores even after contracted periods of exposure, it may not be considered to be "self-sterilizing." How-

- 3 Rosenblatt, M B    The Clinical Manifestations of Amyloidosis, *Ann Int Med* 8 678, 1934
- 4 Walker, G T    Case of Recovery From Amyloid Disease, *Lancet* 2 120, 1928
- 5 Reimann, H A    Recovery From Amyloidosis, *J A M A* 104 1070, 1935
- 6 Wilks, S    Cases of Lardaceous Disease and Some Allied Affections, *Guy's Hosp Rep* 2 Series 3, 105, 1856
- 7 Dixon, H M    Renal Amyloidosis in Relation to Renal Insufficiency, *Am J M Sc* 187 401, 1934
- 8 Perla, D, and Gross H    Atypical Amyloid Disease *Am J Path* 11 93, 1935
- 9 Hunter, W C, and Rush, H P    Amyloidosis of Adrenals as Cause of Addison's Disease, *Ann Clin Med* 5 404, 1926
- 10 Philpott, N W    Addison's Disease in Association With Amyloidosis, *Ann Int Med* 1 613, 1928
- 11 Wild, C    Beitrag zur Kenntnis der amyloiden und der hyalinen Degeneration des Bindegewebes, *Beitr z path Anat u z allg Path* 1 175, 1886
- 12 Steinhilber, F    Ueber eine seltene Form von Amyloid und Hyalinfiltration am Circulations- und Digestionsapparat, *Ztschr f llin Med* 45 375, 1902
- 13 Silwer, H, and Lindblom, A F    Ein Fall von allgemeiner Amyloidose ohne nachweisbare Ursache *Acta med Scandinav* 64 529, 1926
- 14 Larsen, R M    A Pathological Study of Primary Myocardial Amyloidosis, *Am J Path* 6 147, 1930
- 15 Reimann, H A, Koucky, R F and Eklund, C M    Primary Amyloidosis Limited to Tissue of Mesodermal Origin *Am J Path* 11 977, 1935
- 16 Budd, J W    Primary Amyloid Disease of the Heart, *Am J Path* 10 299, 1934
- 17 Lubarsch, O    Zur Kenntnis ungewöhnlicher Amyloidablagerungen, *Virchows Arch f path Anat* 271 867, 1929
- 18 Smetana, H    Experimental Study of Amyloid Formation, *Bull Johns Hopkins Hosp* 37 383, 1925
- 19 Susman, W    Amyloidosis, *Edinburgh M J* 34 527 1927
- 20 Smetana, H    The Relation of the Reticulo-Endothelial System to the Formation of Amyloid *J Exper Med* 45 619, 1927
- 21 Warren, S    Generalized Amyloidosis of the Muscular Systems, *Am J Path* 6 161, 1930

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## OBSERVATIONS ON THE NORMAL EMPTYING TIME OF THE STOMACH OF THE DOG, USING A MIXED MEAL\*

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**I**N 1933 Van Liere and Crisler<sup>1</sup> made a preliminary report on the normal emptying time of the stomach of the dog. During the past two or three years considerable more data has been obtained in the course of experimental work on various conditions influencing the emptying time of the stomach. We feel that these data, combined with those published in the preliminary report, will constitute fairly accurate figures, which may be of aid to other workers studying gastric emptying time, particularly in the dog.

**Methods**—The standard meal used in all of these studies consisted of 40 gm of hamburger, 10 gm of dried ground bread, and 50 cc of milk. Fifteen grams of barium sulphate were added so that the gastric contents could be seen with the fluoroscope. The constituents of the meal were thoroughly mixed, and the animals were fed at the same time each morning. The animals were kept in a quiet room, free from extraneous influences, and were tethered to a chain about

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# THE POSSIBLE IMPORTANCE OF MEDIUMS CAUSING SURFACE AND SUBSURFACE GROWTH OF PATHOGENIC FUNGI TO THE DIAGNOSIS AND TREATMENT OF DISEASE\*

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IN MY work I have found that solid mediums may act consistently in two ways with reference to growth of pathogenic fungi. They may produce a preponderance of either surface or subsurface growth.<sup>1, 2</sup> Workers are familiar with the fact that there is a variable amount of subsurface growth in the case of certain organisms. In mediums of pH 5.6 containing 1 per cent cysteine-cystine as the source of nitrogen and 4 per cent dextrose as the source of sugar, growth is predominantly subsurface, while in similar mediums with peptone substituted for cysteine-cystine, it is predominantly surface. About a month after good subsurface growth had been obtained on cysteine-cystine, in most instances by pouring over the surface a thin layer of Sabouraud's medium, previously subsurface organisms grew on the surface.

The following pathogenic fungi and two nonpathogenic saprophytes, *Lichthemia* sp. and *Scopulariopsis brevicaulis*, were studied: *Achorion schoenleinii*, *Acladium castellani*, *Candida candida*, *Endodermophyton indicum*, *Endodermophyton dermatitidis*, *Epidermophyton cruris*, *Epidermophyton inguinale*, *Epidermophyton rubrum*, *Glenospora Gammeli*, *Geotrichum Bachmann*, *Indiella americana*, *Microsporon Audouini*, *Microsporon felineum*, *Microsporon gypseum*, *Monosporium apiospermum*, *Monilia albicans*, *Oospora humi*, *Sporotrichum Schenckii*, *Trichophyton balcanicum*, *Trichophyton crateriforme*, *Trichophyton decalvans*, *Trichophyton granulolum*, *Trichophyton gypseum*, *Trichophyton gypseum-asteroides*, *Trichophyton gypseum-lacticolor*, *Trichophyton interdigitale*, *Trichophyton japonicum*, *Trichophyton louisianicum*, *Trichophyton niveum*, *Trichophyton purpureum*, *Trichophyton sulfureum*, *Willia anomala*. In order to obtain subsurface growth these organisms were incubated for forty days on mediums consisting either of hydrolyzed hair (Difco Laboratories prepared it for this work) 1 per cent or cysteine hydrochloride Eastman 1 per cent, dextrose 4 per cent, agar 1½ per cent (pH 5.6). The growths were then overlaid with several millimeters of Sabouraud's proof medium and reincubated for forty days. The incubations took place at room temperature in diffused light.

Of the 34 organisms, Sabouraud's medium failed to bring four to the surface: *Endodermophyton tropicale*, *Candida candida*, *Trichophyton balcanicum*, and *Willia anomala*. It is possible that in these instances the organisms were dead or that the medium was too hot when poured over them. In some instances

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## BACTERICIDAL AND BACTERIOSTATIC PROPERTIES OF DIOTHANE SOLUTIONS\*

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SINCE 1930, when the preparation of diothane was first described by Rider,<sup>1</sup> a number of papers have been published treating its chemistry, pharmacology, and clinical uses. Diothane, a local anesthetic, is the hydrochloride of piperidinopropanediol di phenylurethane.

For clinical purposes, diothane is usually employed as a 1 per cent solution, which represents near saturation. Because of its tendency to precipitate from its solutions as the free base, piperidinopropanediol di phenylurethane, it is necessary that considerable care be given to its preparation, particularly with respect to adjustment of pH. The solution must be stored in special non alkaline glass containers preferably of Pyrex, or of similar brand.

Due to the fact that diothane solution is frequently administered by injection, the question of proper means for its sterilization is important. Adequate sterilization may be achieved by boiling, since it has been shown that heating to boiling temperature for moderately prolonged periods of time did not alter its activity as determined by physiologic tests.<sup>2</sup> The degree of decomposition resulting after boiling for periods as long as eighteen hours, as measured by its aniline content,<sup>3</sup> was very slight.

As a result of carrying out sterility tests on diothane solution sterilized by various means, it was soon learned that in 1 per cent concentration it possessed considerable bactericidal activity and that weaker solutions were very strongly bacteriostatic. Further work indicated that this activity was even more pronounced than first described in earlier publications.<sup>4, 5</sup>

When the preliminary work on this phase of the problem was carried out, it was noted that considerable clouding occurred whenever even small amounts of broth culture were added to diothane solutions. It was learned that this was due to the precipitation of the free base as a result of a change in the pH following the addition of the more alkaline broth culture. In preceding papers, it was reported that diothane solutions possessed comparatively low killing powers against certain organisms notably *Staph aureus*. It is now known that this was due, in part at least, to partial inactivation of the diothane solution by the addition of alkaline broth cultures.

Attempts to buffer the various media used in growing the test organisms, or to adjust such cultures after growth took place, proved unsuccessful. In order to prevent subsequent clouding by this means, it was necessary to adjust the pH to a reaction which proved to be incompatible with the growth of the organ-

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# THE QUANTITY OF IODINE IN THE THYROID GLAND OF THE RABBIT AND THE INFLUENCE THEREON OF A DIET OF CABBAGE\*

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THE purpose of the investigation reported here was to establish the amount of iodine in the thyroid gland of the normal rabbit and the proportion of iodine to the weight of the gland, and to compare with this the iodine content of the thyroid glands of rabbits fed for varying lengths of time on a diet consisting almost exclusively of cabbage. At the same time the effect of the cabbage diet on the physical condition as evidenced by body weight was noted.

Various investigators have reported the development of simple goiter in the rabbit apparently as the result of a cabbage diet. Webster<sup>1</sup> recorded some very striking results from which the conclusion is drawn that cabbage grown in certain localities and at certain seasons, particularly during the winter, contains a goitrogenic agent that is much more powerful to produce goiter than is a diet that is deficient in iodine only. The suggestion is made that the cabbage depletes the iodine store of the thyroid gland and that this may be due to a reducing agent acting on the oxidation-reduction systems of the body and causing an increased demand for thyroxin to activate oxidation. McCarrison<sup>2</sup> reported the incidence of goiter as 38.9 per cent in rabbits fed on a mixture of winter cabbage 60 parts, sorghum 20 parts, and whole wheat 20 parts. In rabbits subjected to insanitary conditions and the same imperfect diet as before, he states that goiter developed in 88.9 per cent. As the Edmonton district of western Canada has long been regarded as an area in which goiter, particularly of the myxedematous type, is prevalent, and as cabbage is a much used food, it seemed desirable to study the question of a possible relation between cabbage in the diet and the occurrence of goiter.

For our experiment the diet employed by Webster in the article previously quoted was adopted. This provides for each rabbit: cabbage 250 gm. daily, oats 50 gm. weekly, and hay 20 gm. weekly. Control rabbits were fed on full rations of oats, turnips, carrots and hay. Cabbage was excluded from the food supplied to the control animals. All the food used was grown locally. The animals were caged in pairs and the environmental conditions were the same for all. The total number of animals used was 81, of which 48 were given the cabbage diet and 33 were used as normal controls. The length of time the experiment lasted in individual cases varied from four weeks to twelve. Most of the determinations were made after four-, eight-, and twelve-week periods with a few at the intervening weekly intervals. The procedure was to weigh the animal, to examine it for any evidence of goiter by palpation, and to start it on either the cabbage or the control diet. At the expiration of the predetermined number of

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diothane solution After thoroughly agitating the paper in this solution, transfers were made to sterile broth In all cases the organisms treated in this manner survived exposure equally well for periods of four days, or even longer The culture of *B. subtilis* used in the tests described in Table I contained numerous spores

#### FUNGICIDAL ACTIVITY OF DIOTHANE SOLUTIONS

Information regarding the killing power of diothane solutions against molds and yeasts is desirable Accordingly, its action was tested against a culture of yeast, two strains of *Penicillium* and one of *Aspergillus* Each of these organisms had been isolated from the laboratory and were frequently found as contaminants in other materials

Suitable amounts of the cultures were inoculated in 5 cc quantities of 1 per cent diothane solution and after the intervals noted, transfers were made into sterile broth For the molds, Sabouraud's medium was employed, and for the yeast, dextrose broth was used Readings were made after one week incubation at 25 to 30° C

TABLE II  
FUNGICIDAL ACTIVITY OF DIOTHANE SOLUTION

ORGANISM	TIME OF EXPOSURE IN HOURS						
	1	2	3	4	8	24	48
<i>Penicillium</i> "A"	-	-	-	-	-	-	-
<i>Penicillium</i> "B"	+	+	-	-	-	-	-
Yeast	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	+	+	+	+	-	-	-

It was found that each of the strains of yeast and molds was killed after eight hours' exposure to a 1 per cent diothane solution, the yeast being more readily killed than the others Previous microscopic examination showed that the mold cultures contained numerous spores

#### BACTERIOSTATIC PROPERTIES OF DIOTHANE SOLUTION

In spite of the possession of a rather limited bactericidal activity, it was found that diothane solutions are strongly bacteriostatic In the tests to be described a larger number of organisms was employed, including in the group several pathogens In order to learn something of the possible difference in activity at different pH values each lot of media was divided in two parts, one of which was adjusted to pH of 6.8 and the other to pH of 7.8 As might be anticipated, the greater amount of precipitation occurred in those lots of media having the more alkaline reaction

Beginning with an aqueous solution of diothane of a concentration of 1.200 or 0.5 per cent, various dilutions were prepared by adding calculated amounts to sterile media Inoculations were made directly into the broths thus prepared The tubes were examined for growth after seven days' incubation From those tubes showing no growth, transfers were made into sterile broth in order to determine whether lack of bacterial development in the first tubes indi-

The amount of iodine in the thyroid gland of the normal rabbit averaged 0.033 mg. The maximum quantity in one gland was 0.071 mg. and the minimum 0.013 mg. In the case of the rabbits fed on cabbage, the highest average iodine content, 0.062 mg., occurred in those that had been given the restricted diet for four weeks. The maximum in this group was 0.075 mg. and the minimum 0.037 mg. The rabbits maintained on cabbage for eight weeks showed an average iodine content of the gland of 0.038 mg., with a range from 0.041 mg. to 0.030 mg. Where the cabbage diet was continued for twelve weeks, the amount of iodine in the thyroid averaged 0.031 mg., with 0.062 mg. and 0.011 mg. at the two extremes. It will be observed that the range is almost the same in the two groups, control and cabbage. The maximum quantity found in one gland is almost the same in the two groups and the minimum is likewise practically the same.

The rabbits that had been on cabbage for four weeks yielded the highest average weight for the dried thyroid, the highest average proportion of weight of dried gland to body weight and the highest average total iodine. The average iodine content of the glands of these rabbits is nearly double that of any other group, but when expressed as a percentage of the weight of the gland, it is the lowest of all. It is possible that this is evidence of the existence of goiter and even of a goiter-producing action on the part of cabbage. When it is observed, however, that rabbits subjected to a diet of cabbage for longer periods show thyroid glands that in size, proportion to body weight, and iodine content are practically identical with those of animals maintained on a full diet, it seems necessary to conclude that, even if the cabbage was a causative agent in the departure from normal observed, the disturbance was a temporary one.

TABLE II

	AVERAGE BODY WEIGHT AT DEATH GRAMS	AVERAGE WEIGHT OF THYROID ON REMOVAL MILLIGRAMS	PROPORTION OF THYROID TO BODY WEIGHT %	AVERAGE WEIGHT OF THYROID DRIED MILLIGRAMS	PROPORTION OF DRIED THYROID TO BODY WEIGHT %	AVERAGE TOTAL IODINE MILLIGRAMS	PROPORTION OF I TO WEIGHT OF DRIED THYROID %
<i>Controls</i>							
4 wk.	2260.0	622.5	0.028	100.2	0.0044	0.033	0.033
8 wk.	2033.0	773.0	0.038	113.7	0.0056	0.032	0.028
12 wk.	2417.0	300.0	0.012	92.5	0.0038	0.035	0.038
<i>Cabbage</i>							
4 wk.	2271.0	1785.3	0.079	512.0	0.0226	0.062	0.012
8 wk.	2003.0	1435.7	0.072	106.9	0.0053	0.038	0.036
12 wk.	1806.0	569.3	0.032	117.7	0.0065	0.031	0.026

## SUMMARY

1. The average amount of iodine in the thyroid gland of the normal rabbits studied was 0.033 mg., the average weight of the dried gland was 101.6 mg., and the iodine content equalled 0.033 per cent of the gland by weight.

ever, from a consideration of the foregoing results it is at once apparent that a 1 per cent solution of diothane, once sterilized by boiling, is well preserved and will remain sterile if ordinary aseptic technique is observed during the removal of small amounts of the solution from its container.

It is reasonable to assume that such bacteria, with the exception of spore bearers, as may become accidentally introduced into the solution will be killed within an hour or so. The results with the molds indicate that a longer period of exposure will be required to kill them.

#### SUMMARY

1 Diothane solution in concentration of 1 per cent is strongly bactericidal against the more common contaminants with the exception of *B. subtilis* and probably other spore formers.

2 In concentration of 1 per cent diothane solution will kill a resistant strain of *Staph. aureus* within one hour, and *B. typhosus* and *B. coli* within five minutes.

3 The spores of *B. subtilis* will resist the activity of 1 per cent diothane solution for four days or longer.

4 *B. subtilis* is inhibited in broth containing diothane in concentration as low as 1:3200.

5 Molds and yeasts are killed by diothane solutions.

6 While strongly bactericidal against many organisms, diothane solution may not be considered to be "self sterilizing" because of its weak action against *B. subtilis* spores.

#### REFERENCES

- 1 Rider, T. H. and Hill, A. J. Studies of Glycidol. I. Preparation From Glycerol Monochlorohydrin, *J. Am. Chem. Soc.* 52: 1521, 1930.
- 2 Rider, T. H. Piperidinopropenediol Diphenylurethane Hydrochloride, a New Local Anesthetic, *J. Pharmacol. & Exper. Therap.* 47: 255, 1933.
- 3 Cook, D. S., Bambrich, K., and Rider, T. H. The Stability of Diothane Solution II, *J. Am. Pharm. A.* 24: 269, 1935.
- 4 Rider, T. H. Pharmacological and Preliminary Clinical Observations on Diothane, a New Local Anesthetic, *Anesth. & Analg.* 12: 259, 1933.
- 5 McKim, G. I., Smith, Parke G., Rush, T. W. and Rider, T. H. Use of Diothane as a Local Anesthetic in Urology, *J. Urol.* 29: 277, 1933.

The number of pollen grains collected during this interval was determined by counting the grains contained in an area one inch square on the slide. Daily logs of the pollens collected at the different stations were kept and charts of the findings made.



Chart 1

Chart 1 is a relief map of the city of San Diego and its immediate environs. The locations of the four pollen collecting stations are indicated on the map.

Chart 2 compares the total pollen counts from May to November, 1934, at the four stations. At all stations the slide facing west contained the greatest number of pollen grains with rare exceptions; the slides facing south, north, and east contained the next largest numbers in the order named except that during

the growths seemed almost as profuse as on Sabouraud's proof medium. This, however, is not to be expected since the liver of Sabouraud's is only a few millimeters deep.

The ability of the Sabouraud's proof medium to attract organisms from the subsurface suggests its potential use in packs to be placed on areas of chronic disease with several objects in view. First, it might serve to attract the etiologic agent to the surface. Second, if an antiseptic were incorporated in it, it might not only attract to the surface but kill. Third it might attract organisms to the surface so that in areas from which cultures have been previously negative, diagnosis might be made. When made, the applications should be kept moist either by covering them with adhesive or other means. Either the liquid medium can be used, or the solid medium incorporated in small gauze sponges. The mediums should be sterilized.

It is possible that other mediums than Sabouraud's might be more proficient. Sabouraud's in my experience however produces a special type of growth as any.

The difficulty with reference to antiseptics where chronic lesions are below the superficial layers is apparent. Any method which would efficiently dislodge organisms located deep might prove effective by allowing lethal antiseptics to contact them.

The cysteine cystine mediums allow subsurface growth to a depth of usually not more than 1 cm.<sup>1, 2</sup> The depth with reference to proportional concentrations of cysteine cystine in the skin may help explain chronicity in skin diseases. With reference to bacteria our results have not been enlightening.

#### REFERENCES

- 1 Williams, J. W. Invasiveness of Skin Infections Caused by Pathogenic Fungi and Subsurface Mycelium, *Science* 83: 296, 1916.
- 2 Idem. Subsurface Growth of Pathogenic Fungi on Hair, Pig Skin and Cysteine Cystine Mediums, *Arch. Dermat. & Syph.* In press.

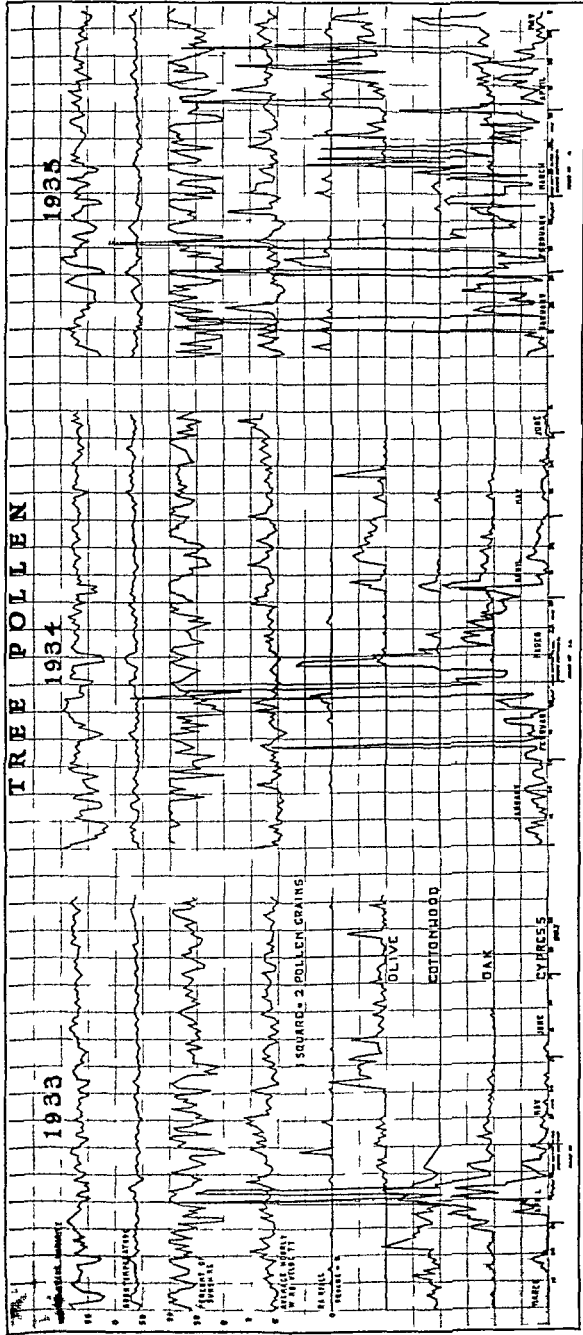


Chart 5

weeks the animal was weighed, killed rapidly by the inhalation of chloroform vapor, and the thyroid removed. The gland was dried between filter papers for five minutes and weighed. It was then dried at a temperature of 100° C for forty-eight hours and reweighed. Subsequently all the glands were dried again in the oven as before, weighed and the iodine content of each was determined.

It might be noted that as a preliminary measure tests were made as to the best way to kill the rabbits. It was found in every case where the neck blow was employed that all the tissues of the neck including the thyroid gland, were thereby rendered congested. The gland was usually found engorged with blood when death was produced by this means but this was not the case when chloroform was used.

## RESULTS

The control animals showed a gain in weight up to eight weeks, but those kept for twelve weeks showed an insignificant loss in weight. The cabbage rabbits, on the contrary, showed marked loss of weight (Table I). All the rabbits used were young adult animals and litters were divided, so far as possible, between the two main groups. In view of the other findings, this loss of weight is to be regarded simply as evidence of inanition.

TABLE I

	CONTROLS				CABBAGE		
	AVERAGE BODY WEIGHT				AVERAGE BODY WEIGHT		
	GRAMS		GAIN OR LOSS %		GRAMS		GAIN OR LOSS %
	BEGIN	END			BEGIN	END	
4 wk	2010.0	2200.0	+12.4	4 wk	2037.0	2271.0	+10.4
8 wk	1612.0	2070.0	+26.1	8 wk	2122.0	2003.0	-5.6
12 wk	2461.0	2417.0	-1.8	12 wk	2398.0	1806.0	-24.7

Little importance can be attached to the weight of the fresh thyroid. The amount of water in the gland is very variable. This is exemplified by our records of the individual glands which show a loss of weight when dried from a minimum of 50.2 per cent to a maximum of 80.9 per cent in the control group and from 57.4 per cent to 84.3 per cent in the cabbage group. When the weight of the thyroid gland that has been dried is compared with body weight, it is found to constitute from 0.0038 per cent to 0.0065 per cent in all groups, cabbage and control, except the one comprising animals maintained on cabbage for four weeks. In this group the average weight of the dried thyroid and its proportion to body weight are much greater than in any other group, cabbage or control. This is due almost entirely to one gland, which had a weight when dried of 1,504.9 mg.

The method employed to determine the iodine content of the thyroid glands analyzed was described by Remington, McClendon, von Kolnitz and Culp<sup>3</sup>. In connection with these analyses the following comment should be made. A source of error in the colorimetric determination of iodine is the variation of the ratio of iodine in the carbon tetrachloride to the iodine in the water. The amount of iodine remaining in solution in the water varies with the concentration of salts in the water. In these analyses the concentration of salts varied with each run and, since the effect of the variation was not determined, an error was introduced.

## VARIATIONS IN THE BLOOD CHOLESTEROL OF MAN OVER A TIME PERIOD\*

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IT HAS been pointed out from time to time that single duplicate estimations of any chemical compound or element in the body fluids are not representative; that under various conditions, artificially or naturally induced, the chemical equilibrium of the body alters itself, this state of affairs occurring even under basal conditions.

Thus, the lipids in the blood of man and animals have, in general, been considered to be variable, such variability being strongly altered by the ingestion of food. These statements have their origin in work done years ago, for instance, by Ahlfeld<sup>1</sup> who noticed that the serum of an animal becomes definitely milky after a meal rich in fat. In more recent years, while it has been shown that such a lipemia occurs in carnivorous animals after intensely fat meals,<sup>2-5, 14</sup> it has not been demonstrated to be true for herbivorous animals, and practically not at all for man.<sup>6-11</sup>

Man and Gildea,<sup>12</sup> evidently feeling that in these experiments the relationship between the weight of the individual and the weight of the fat administered had been neglected, noted that an alimentary lipemia could be produced in man, provided 3.5 gm. of fat per kilo of body weight be given. They also found that a smaller ration containing about 0.5 gm. of fat per kilo resulted in variations which were unreliable. Boyd<sup>13</sup> pointed out that these experiments indicated that well over 200 gm. of fat must be taken by a man at one meal in order that an alimentary lipemia might be produced; and since a normal person under normal conditions and doing a moderate amount of muscular work consumes much less than 200 gm. of fat at one meal, or at three meals for that matter, it was highly improbable that in such a man an alimentary lipemia ever occurred under normal conditions. Then Boyd proceeded one step further and in attempting to discover whether or not there was any variation in the concentration of blood lipids in man during a twenty-four-hour period, found but slight variations. The deviation per person per day for free cholesterol was 6.5 per cent and for ester cholesterol, 7.2 per cent. The variation in blood cholesterol from one person to another per twenty-four-hour period he found to be two to three times as great as the average variation per person per day. Burger and Somach<sup>6</sup> obtained similar results for total cholesterol in the whole blood of man during a similar period.

Thus, for a man consuming three ordinary meals per day, there is no existing evidence that any marked alteration in the blood cholesterol would be found for any given twenty-four-hour period. It is the purpose of this paper to extend

\*From the Psychiatric Clinic, Boston State Hospital.  
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2 The average amount of iodine in the thyroid gland of rabbits kept on a diet of cabbage for four weeks was 0.062 mg., the average weight of the dried gland was 512.0 mg., and the iodine content equaled 0.012 per cent of the gland by weight.

3 Rabbits maintained on a diet of cabbage for periods of eight and twelve weeks had thyroid glands that in size and amount of iodine contained were the same as those of the control animals.

4 Rabbits kept on a diet consisting almost exclusively of cabbage invariably lost weight.

The iodine determinations reported in this paper were made by Mr. T. L. Cairns, whose assistance is gratefully acknowledged.

Our thanks are tendered to the Carnegie Corporation of New York for a grant to defray the expenses of this research.

#### REFERENCES

- 1 Welster, B. Studies in the Etiology and Nature of Simple Goiter as Produced Experimentally in Rabbits. *Trans. Am. Assn. Study Goiter* p. 12, 1921.
- 2 McCarrison, P. Iodine and Goitre. *Brit. M. J.* 2 (1) 1922.
- 3 Remington, R. I., McClenlon, J. I., von Kunitz, H. and Culp, F. B. The Determination of Traces of Iodine. IV. Iodine in Small Quantities of Thyroid and Other Tissues, *J. Am. Chem. Soc.* 52, 980 (1930).

## THE POLLEN CONTENT OF THE AIR OF SAN DIEGO, CALIFORNIA\*

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WITH THE TECHNICAL ASSISTANCE OF MRS. HELEN McMICHAEL

**A** KNOWLEDGE of the pollen content of the air of a given locality is essential for the intelligent study and treatment of allergic diseases associated with pollen sensitization in that locality. This is particularly true of Southern California because of the diversity of vegetation and the influence upon it of the climatic conditions peculiar to that part of the country.

In order that we might have a basis upon which to work in the treatment of these conditions in San Diego, a study of the pollen content of the air of that city, extending over a period of two and one half years was made. Slides for the collection of pollen grains were exposed each day at the Clinic station during this time. From May 1, 1934 to Nov. 1, 1934 pollens were collected at three additional stations in the city, these stations representing, as far as possible, the varying topographical characteristics of the different sections of the city. At each station, four slides were placed in upright position, each slide facing one of the four points of the compass so that pollen was collected from all directions. A shelter to protect the slides from rain but open on the four sides was provided. Each set of slides was exposed for twenty-four hours.

\*From the Rees Stealy Clinic.

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TABLE I  
TOTAL CHOLESTEROL IN MG. PER 100 C.C. WHOLE BLOOD

TIME IN WEEKS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	MEAN	DEVIATION FROM MEAN		RANGE	GENERAL MEAN
																		MAX.	MIN.		
Case 1	172	173	183	187	136	105	178	140	130	150	137	123	125	130	125	100	143	+44	-3	100-187	147 (Range of Mean Values +13 to +0)
Case 2	158	186	166	186	151	165	166	162	178	156	172	160	175	165	170	165	167	+19	-1	151-186	
Case 3	131	110	111	123	148	136	122	110	106	125	123	110	126	120	125	130	122	+26	+0	106-148	
Case 4	188	198	180	146	182	150	146	155	167	160	143	162	157	142	152	142	160	+38	+0	142-198	
Case 5	148	138	136	120	131	130	120	136	123	126	132	133	137	120	140	132	132	+19	+0	120-151	
Case 6	185	154	172	115	171	155	156	140	150	130	157	142	160	150	155	155	152	-37	+2	115-185	
Case 7	155	145	157	150	170	140	135	145	148	143	154	153	145	140	145	150	148	+22	+0	135-170	
Case 8	144	186	153	160	190	150	134	147	158	143	150	160	140	148	170	144	154	+36	-1	134-190	
Case 9	159	150	131	170	120	146	147	137	130	144	140	150	136	124	133	140	141	+29	-1	124-170	
Case 10	170	165	152	116	136	167	196	170	164	157	153	152	143	148	140	154	155	+41	-1	116-196	

the winter months there were frequently more pollen grains on the slide facing north than on the one facing south. As might be expected, the pollen content of the air was lowest at Sunset Cliffs which is situated on the ocean. It is interesting to note that the count was greatest at the stations located in the central (Clinic) and southern (Logan Heights) portions of the city, even though the western (State College) station is considerably farther inland.

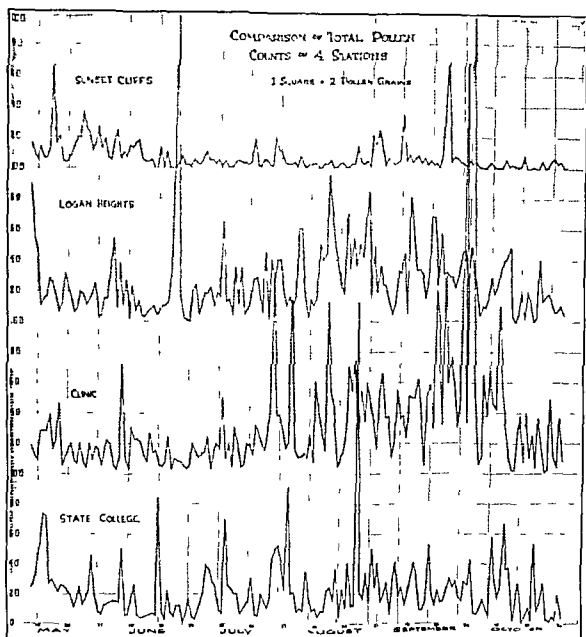


Chart 2

Charts 3 and 4 show the daily total pollen counts at the Clinic for the morphologically distinct groups of pollens, and the daily hourly wind velocity, rainfall, noon temperature, percentage of sunshine and relative humidity for the city for the years 1933, 1934, and 1935 (to May, 1935).

Chart 5 gives the pollen counts of the cypress, oak, cottonwood, and olive trees for the years 1933, 1934, and 1935 (to May, 1935) at the Clinic station.

Knowledge of certain meteorologic data concerning San Diego is essential to the intelligent understanding of these charts. The daily variations are recorded graphically. They may be summarized and amplified as follows:

## REFERENCES

1. Ahlfeld, F.: *Zentralbl. f. Gynäk.* 1: 265, 1877.
2. Bloor, W. R.: *J. Biol. Chem.* 23: 317, 1915.
3. Bloor, W. R.: *J. Biol. Chem.* 24: 447, 1916; *Physiol. Rev.* 2: 92, 1922.
4. Greenwald, L.: *J. Biol. Chem.* 21: 29, 1915.
5. Reicher, K.: *Verhandl. Kong. inn. Med.* 28: 327, 1911.
6. Burger, M., and Somach, I.: *J. Biol. Chem.* 97: 23, 1932.
7. Chaikoff, I. L., McGavack, T. H., and Kaplan, A.: *J. Clin. Investigation* 13: 1, 1934.
8. Gardner, J. A., and Gainsborough, H.: *Biochem. J.* 22: 1048, 1928.
9. Hiller, A., Linder, G. C., Lundsgaard, C., and Van Slyke, D. D.: *J. Exper. Med.* 39: 931, 1924.
10. Page, I. H., Pasternak, L., and Burt, M. L.: *Biochem. Ztschr.* 223: 445, 1930.
11. Rony, H. R., and Levy, A. J.: *J. LAB. & CLIN. MED.* 15: 221, 1929.
12. Man, E. B., and Gildea, E. F.: *J. Biol. Chem.* 99: 61, 1932.
13. Boyd, E. M.: *J. Biol. Chem.* 110: 61, 1935.
14. Bloor, W. R.: *Physiol. Rev.* 2: 92, 1922.
15. Schube, P. G.: *J. LAB. & CLIN. MED.* 18: 306, 1932.
16. Schube, P. G.: *Am. J. Psychiat.* 12: 355, 1932.

## STUDIES IN BACTERIOPHAGE\*

### III. THE SIGNIFICANCE OF TESTS FOR THE INHIBITION OF THE BACTERIOPHAGE PHENOMENON BY HUMAN SERUM

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## INTRODUCTION

OUR combined clinical and laboratory experience with bacteriophage has shown us clearly that there is a definite relationship between the presence or absence of lysis in the test tube and the favorable or unfavorable action of bacteriophage in the patient.<sup>1, 2</sup> The treatment of certain cases yielding organisms resistant to bacteriolysis met with complete failure, while those which yielded susceptible cultures generally responded favorably. However, some of these cases failed to respond to the phage treatment. In an attempt to explain the discrepancies, we have studied the effect of the different substances, such as serum, pus, tissue extracts, etc., with which bacteriophage comes into contact when introduced into the lesion. The present paper concerns itself with the inhibition of bacteriophage by serum.

## LITERATURE

Gratia<sup>3</sup> in 1921 observed that human serum contains a substance which neutralizes the lytic effect of bacteriophage on bacteria in the test tube and offered this fact as the possible explanation of certain failures in phage therapy. In 1925, however, Wolff<sup>4</sup> failed to confirm Gratia's findings. On the other hand, in 1928, Rosenthal<sup>5</sup> reported that he had found that the serum of patients suffering from recurrent furuncles or severe carbuncles, contained an inhibiting substance, demonstrable in dilutions as high as 1-2000, which was not found in the

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1. The mean annual precipitation is about 16 inches, the rains being confined almost exclusively to the winter months. Maximal variations in amount from year to year are common.

2. The prevailing direction of the winds is from the ocean about 90 per cent of the time. The direction of winds also is changing during the winter season.

3. The hourly wind velocity is rarely less than 5 or more than 15 miles per hour.

4. The mean annual temperature is about 60° F. and annually 13° F. However, diurnal variations of as much as 20° F. may occur. Killing frosts are unknown.

5. The relative humidity averages 75 per cent.

Undoubtedly the most important factor determining the pollen crop in a locality for a given year is the amount of rainfall during the growing season. This is shown by the corresponding differences in rainfall and in pollen crops for the years 1933-1934 and 1934-1935. The amount of rainfall in the winter of 1933 and 1934 was about 5 inches, whereas in the winter of 1934 and 1935 it was about 15 inches. The rainfall in the first half of 1935 was approximately double that of the corresponding half of 1934. The pollen concentration of the air during the long and dry winter of 1934 to a certain extent by the pollen from some of the arborescent plants which flowered late in the dry or irrigation for moisture.

Owing to the equable climate factor in San Diego was one of the various species and has sharply demonstrated a marked localism in which marked seasonal variations in climate of the West. The flowering dates of the same plant may vary widely in different years. For example, the pollinating season for the cedar started in March in 1933 and in January in 1934 (Chart 5). Furthermore, pollen of various species are in the air long after the known pollinating periods of these species have ended. This is undoubtedly explained by the fact that, due to the absence of rainfall during the summer, pollen is not washed out of the air. Also, that which falls on the leaves of the plants may remain there until it is dislodged by a gust of wind, thus disseminating more pollen into the air.

The amount of pollen from a given plant may vary markedly from year to year. This point is illustrated by the fact that the pollen from the cedar was very plentiful in 1933 and relatively small in amount in 1934 and 1935. The pollen count of the cypress on the other hand was low in 1933, high in 1934 and very high in 1935 (Chart 5).

If the actual pollen concentration of the air in this city were compared with the extremely high values which obtain in any midwestern city, it might be assumed that pollinosis is a negligible problem here. This is far from the truth as many sufferers who have lived in both localities will attest. The lack in pollen concentration is made up to a large extent by the prolongation of the season and the diversity of vegetation. Allergic diseases associated with pollen sensitization is a perennial disorder in San Diego.

I wish to express my appreciation to Dr. George F. Hart, formerly with the Allergy Clinic of the University of Chicago, and now associated with the Clinic of the Mayo Institute of this paper.

12. Raiga, A.: Guérison d'une arthrite purulente de genou à Streptocoque et tétragène par une inoculation intra-articulaire de bactériophage, *Bull. et mém. Soc. de chir. de Paris* 26: No. 12, 1934.
13. Riglieri, P., and Fischer, I.: Contribution à l'étude des propriétés anti-bactériophagiques des sérums humains, *Compt. rend. Soc. de biol.* 108: 674, 1931.
14. Applebaum, M., and MacNeal, W.: The Influence of Pus and Blood on the Action of Bacteriophage, *J. Infect. Dis.* 49: 225, 1931.
15. Gratia, A., and Mutsaers, W.: L'action inhibitrice du sérum normal sur la lyse du staphylocoque doré par les bactériophages staphylococciques polyvalents, *Compt. rend. Soc. de biol.* 106: 943, 1931.
16. Mutsaers, W.: De l'action protectrice exercée par le sérum normal sur les staphylocoques dorés contre la fixation du bactériophage, *Compt. rend. Soc. de biol.* 108: 235, 1931.
17. Colvin, M. G.: Behavior of Bacteriophage in Body Fluids and in Exudates, *J. Infect. Dis.* 51: 527, 1932.
18. Bulgakov, N. A.: Personal Communication.

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## THE DOCTOR AS SCHOLAR

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THE contributions of the medical man to pure scholarship have been of the highest order. Together with the doctor of divinity the doctor of medicine helped to keep the flame of learning alive during the Middle Ages, and in the centuries following added much to its luster by many significant contributions to almost every branch of pure scholarship.

Among the first of the medical scholars was Thomas Linacre who lived from 1460 to 1524. He began his education at Oxford University and continued it at the ancient University at Padua, Italy, from which he received his degree in medicine. Upon his return to England he distinguished himself as a physician, and when Henry VIII became king, Linacre was appointed as one of his physicians. Dr. Linacre enjoyed a large medical practice in London and numbered many prominent persons as his patients.

But medicine was not the only thing that interested Linacre. He was very much interested in religion and received priest's orders. He then restricted his medical work and devoted more of his time to scholarship. He was the founder of the College of Physicians which was incorporated in 1518. Linacre was its first president, and he retained this office until his death. He aided the Universities of Oxford and Cambridge in scholarly research as well as in the matter of monetary gifts. He was one of the first of the great English scholars.

A contemporary of Linacre was John Caius (1510-1573) who was born at Norwich and studied at Gonville Hall, Cambridge. Like Linacre he went to Padua to round out his education. Here he came in contact with the leading medical lights of the time, Montanus in medicine and Vesalius in anatomy. He became a very close friend of the great anatomist and lived in his house for eight months.

His career seemed to parallel that of Linacre with remarkable fidelity. Upon his return to England he became physician to Edward VI and later to Queen Mary. His practice was enormous and he became wealthy. He never married, and for this reason determined to devote his wealth for the encouragement of scholarship. In 1557 he obtained royal permission to refound Gonville

this knowledge one step further, i.e., to determine the variation of blood cholesterol in man consuming three ordinary meals per day over a series of weekly periods. The importance of clarifying this aspect of the problem of cholesterol metabolism is self evident.

#### PROCEDURE

The individuals used in this work were on a fairly standardized hospital diet. All were males. The age range was eighteen to thirty five years. The body habitus was clinically determined as pyknic 5 and asthenic 5. The bloods were obtained in the morning, once a week and while the individuals were in a fasting state. The method of estimation of the blood cholesterol was a modification of the Myers-Waidell<sup>14</sup> in which the total cholesterol was determined on whole blood. The procedure was carried out upon the blood immediately upon its withdrawal. Each cholesterol estimation was performed in duplicate, the average result being used in each instance and expressed as milligrams of cholesterol per 100 cc of whole blood. The normal range for this method as determined by Schube<sup>16</sup> and taking into consideration the error of the method, is 100 to 200 mg of cholesterol per 100 cc of whole blood.

#### RESULTS

The cholesterol estimations were performed upon 160 samples of whole blood, one sample per week on ten individuals, over a period of four months. The changes in the concentration of the cholesterol in each person is shown in Table I. The total range, mean value, and the maximum and minimum deviation from that value are likewise shown in Table I.

The total cholesterol in the whole blood for the group of cases varied from 100 to 198 mg with a mean value of 147 mg. This range and mean are entirely within the limits of normal for the method used, as Schube<sup>16</sup> has shown. Over the time period of four months used in this study, the blood cholesterol in the individual case was found to fluctuate in quantity somewhat. This fluctuation in successive weeks may be marked (73 mg in Case 1, sixth to seventh week) or practically immeasurable (1 mg in Case 10, eleventh to twelfth week). When the fluctuation of cholesterol is studied in the individual case as related to the mean value in that case, the fluctuation is less marked (from a maximum of +44 to -3 mg to a minimum of +19 to  $\pm 0$ ). If these values can be taken as representative, then it can be said that in the individual case the total blood cholesterol can vary in quantity from week to week when the differences of the successive values are studied, over a range of 0 to 73 mg, or over a lesser range of 19 to 47 mg if each value is studied in relation to its individual mean value. Such variations still permit the cholesterol to remain within the limits of normal, for in no instance, regardless of fluctuation, did the blood cholesterol deviate from the range which was normal for the method.

#### DISCUSSION

This study exhibits several interesting facts. (a) When a large group of total cholesterol is estimated over a number of weeks in the bloods of normal individuals, the range of these values and the mean is within the normal range

One of the earliest contributions to literary scholarship was made by Robert Watt (1774-1819). He was born on a farm near Stewarton in Ayrshire and graduated in arts at Glasgow. For awhile he acted as parochial schoolmaster, but he was very much interested in medicine and returned to Glasgow to enter upon his medical studies. In 1799 he became a Licentiate of the Faculty of Physicians and Surgeons. In the same year he began general practice in Paisley where he remained for eleven years. In 1810 he obtained his M.D.

Dr. Watt was a promoter and the first president of the Glasgow Medical Society. In 1816 he was elected president of the Glasgow Philosophical Society, and in the following year his health became so impaired that he withdrew from medical practice.

He then left Glasgow for Campvale where he entered upon his studies in literature. Here he conceived and carried out his great work the *Bibliotheca Britannica*. His health grew steadily worse and he died in 1819, with the *Bibliotheca* practically completed.

Dr. Watt's great achievement, the *Bibliotheca Britannica; or a General Index to British and Foreign Literature*, was completed five years after his death. It was a monumental achievement for one man. It is the first great bibliographic work of modern times. It consists of four large quarto volumes. The first two contain the names of more than 40,000 authors, with brief summaries of their works. The second part contains all the books mentioned in the first part, arranged under subjects.

Another doctor who contributed much to literary scholarship was Peter Mark Roget (1779-1869), the son of a French pastor in London. He graduated in medicine at the University of Edinburgh at the early age of nineteen, but he continued his studies in London.

In the year 1805 Dr. Roget was appointed a physician to the Manchester Infirmary, and in this capacity he also became one of the founders of the Manchester Medical School. Three years later he removed to London where he engaged not only in a very extensive medical practice but in other activities as well.

In between office consultations and calls at his patients' homes, Dr. Roget made a very thorough investigation of the water supply of the metropolis at the request of the government. He also invented a logo-logarithmic slide rule which won for him election to the Royal Society. In time he succeeded Sir John Herschel as secretary of this society. During this time he also wrote a Bridge-water treatise on *Animal and Vegetable Physiology Considered With Reference to Natural Theology*. This work attained great popularity and went through many editions. Dr. Roget was also one of the founders of the University of London.

The general public remembers Dr. Roget not for the many accomplishments during his lifetime, but for one single literary production. This is the world-famous *Roget's Thesaurus of English Words and Phrases*. He wrote this book after 1840 when he retired from professional practice to devote himself to this work exclusively.

*Roget's Thesaurus* went through 28 editions during his lifetime, and what edition it has reached at this date, almost a hundred years after it was first published, I do not at the moment know. It has been a steady seller and is still as popular as ever. There is not a writer or editor of English anywhere in the world who has not heard of this work or who has not a copy on his desk.

and the mean range for total blood cholesterol as estimated by the given method (b) When the total blood cholesterol is estimated and considered only in the individual case over a series of weekly intervals, the range continues within the limits of normal but the mean value for the individual may vary quite a bit (c) When the range of total cholesterol in individual cases is compared, it is found that these individual ranges may vary markedly, although remaining within normal limits (d) When the means of the blood cholesterol of the individuals are compared, it is found that there is a range of variation of 35 mg (e) The age of the individuals in this series apparently did not affect the cholesterol, and (f) no correlation could be established between the body type and cholesterol nor variation of cholesterol. It is fully realized that for (e) and (f) the number of cases is small and that a larger number might show something of significance. It is the purpose here to present the factual observations.

Since it has been rather conclusively shown by Man and Gildea that in order to produce an alimentary lipemia in excess of 200 gm of fat must be taken by an individual at one meal and since these individuals certainly never approached this figure in a total of three meals per day, it is highly improbable that the lipin in the food had anything to do with the variations observed. Furthermore, on a diet such as the above Boyd and Burger and Somach found that an increase in blood cholesterol which is significant does not occur. Therefore, such variations as have been found will have to be explained in some other way.

The difference in quantity of blood cholesterol in different normal individuals at the same time can only be explained at the present on the basis of individual peculiarities in handling the cholesterol in the blood, even though it be within normal limits at all times. The fluctuations in the blood cholesterol from week to week can be explained at the present only upon the same basis. It is highly probable that the ebb and tide of the blood cholesterol depend upon the needs of the body for it and upon the fluctuating ability of the organs of excretion to retain it in the blood stream. These, however, are merely conjectures and should, in our present state of knowledge of cholesterol metabolism, be regarded as such.

#### SUMMARY

In this study it has been shown that over a time period involving weeks

- a The blood cholesterol in different individuals fluctuates along different patterns
- b The blood cholesterol from week to week can change over a range of 0 to 73 mg
- c The blood cholesterol when considered in relation to its individual mean over a long time period fluctuates over a range of 19 to 47 mg
- d There is certain ebbing and flowing of the blood cholesterol which is always within normal limits, which cannot be explained by any known mechanism, but which must be attributed to unexplained occasional changes in the fat metabolism of this or that person

# LABORATORY METHODS

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## A PRECISE SILVER IMPREGNATION METHOD FOR BLOOD CELLS\*

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MANY problems in hematologic morphology and differentiation remain unsolved because of limitations in our technical methods. Similar problems in other fields of histology have been cleared up by the use of silver impregnation methods. It is, therefore, surprising to find that silver impregnation has had only limited application in the field of hematology.

Rhinehart<sup>1, 2</sup> impregnated erythrocytes with silver and studied their hemoglobin content. His method also is suitable for the impregnation of nucleated blood cells, but the technique is too complicated to lend itself to wide application. Dawson<sup>3</sup> applied the formulas devised by Cajal and da Fano, to the study of the reticular granules and segregation apparatus of erythrocytes. These methods, however, are not sufficiently exact to yield standard results. Tomita, Kawabe and Yuba<sup>4</sup> and Perez Ara<sup>5</sup> also used silver to impregnate blood cells, but their reports are not available to me. The following method has been in use for some time. It is relatively simple and yields uniformly precise results.

### METHOD

*Preparation.*—Blood films, bone marrow smears or “touch preparations” from marrow, spleen, or lymph nodes are made in the usual manner and dried in air. These may be stored for an indefinite period, if kept free of dust, without impairment of the subsequent impregnation. Because cover slips can be stored in small cardboard containers which are easily numbered, it is more convenient to make the smears on cover slips than on slides.

*Fixation.*—The preparations are fixed in 10 per cent formalin (4 per cent formaldehyde) which need not be neutralized. Fixation is complete in a few minutes and is followed by a wash in water.

*Mordanting.*—The fixed smears are mordanted in 2.5 per cent aqueous solution of ferrous ammonium sulphate (iron alum) for ten minutes. Mordanting may be prolonged almost indefinitely without apparent interference with the subsequent impregnation. The mordant is followed by thorough washing in four changes of distilled water.

*Coating.*—The cover slips are dipped in 1.0 per cent aqueous solution of gelatin, to 50 c.c. of which are added two drops of 2 per cent aqueous sodium carbonate. This protects the smears during impregnation and prevents the deposition of diffuse silver precipitate while the preparations are in the silver

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serum of persons free of infection. Later, Rosenthal<sup>6</sup> found that results obtained when he mixed serum with phage first and then added the bacteria were often different from those obtained when he mixed the serum with the bacteria first and subsequently added the phage. Inhibition of lysis sometimes took place by one method and not by the other. He concluded from these tests that in the first instance the serum directly neutralized the lytic agent while in the other it indirectly inhibited the lysis by rendering the bacteria resistant. The first effect he named "direct antiphage," the second, "indirect antiphage." Rosenthal stressed the importance of direct antiphage and suggested "autohemotherapy," stating that this caused the direct antiphage to disappear from the blood. However, he did not present any experimental data in substantiation of this statement.

In 1928, Raiga<sup>7</sup> reported 4 cases of failure of bacteriophage treatment in patients whose organisms were susceptible to the phage in the test tube, but whose serums were found to contain an inhibiting substance active in dilutions up to 1:2000.

In 1930, Raiga<sup>8</sup> enlarged upon his previous report and attempted to correlate the results of his therapy with the presence of the antiphage. This author followed Rosenthal in distinguishing two kinds of antiphages calling them the antiphage of bacterial resistance (indirect of Rosenthal) and the antiphage of bacteriophage inactivation (direct of Rosenthal). After correlating his tests with the clinical results, Raiga, in contradistinction of Rosenthal, concluded that the antiphage of bacterial resistance (indirect) was of importance while the antiphage of bacteriophage inactivation (direct) which he encountered in practically every case, was of no significance. Beside the staphylococcus antiphage of bacterial resistance, Raiga found that many serums also had hemolytic streptococcus antiphage, which seemed to him to have considerable clinical importance. Raiga did not state his method for determining the presence of these antiphages in this report, but later publications indicate that he used the method of Rosenthal at the beginning of the study and that of Sertie later.<sup>9, 12</sup>

In 1931, Righieri and Fischer<sup>13</sup> studied 80 serums of surgical patients and found staphylococcus antiphage in every case.

In the same year, Applebaum and MacNeal<sup>14</sup> reported that purulent exudates, blood, and blood serum exerted a marked inhibitory influence on the lytic action of the staphylococcus bacteriophage. These authors were the first to suggest that any given serum might alter the lytic effect of phage in varying degrees when placed in contact with different strains of bacteria.

In 1931, Gratia and Mutsaers<sup>15</sup> and Mutsaers<sup>16</sup> extended their work and found that while serum had an inhibitory action on the phage, this action was usually transient, for lysis frequently took place in the second twenty-four hours. They also observed that a serum which prevented the action of the phage on *Staphylococcus aureus*, did not affect the action of phage on *Staphylococcus albus*.

Colvin<sup>17</sup> (1932) found that serum would sometimes inhibit the action of phage in high dilutions when the concentrated serum failed to inhibit.

How may the variable results of these different authors be reconciled? Differences in technique undoubtedly explain most of these variations, for there was no uniformity in the potency of the phages, the age or concentration of serum,

bath. The excess of gelatin is allowed to drain off and the cover slips are briefly dipped in distilled water. This washes away some of the gelatin, leaving only a thin film.

*Impregnation.*—The diammoniosilver hydroxide solution of Kubie and Davidson<sup>6</sup> diluted with an equal amount of distilled water is employed:

To 5 c.c. of 10.2 per cent aqueous solution of silver nitrate, add strong ammonium hydroxide solution until the precipitate of silver hydroxide is just dissolved. Then add 5 c.c. of 3.1 per cent aqueous solution of sodium hydroxide and just dissolve the resultant precipitate with a drop or two of strong ammonium hydroxide. Dilute the diammoniosilver hydroxide solution to 100 c.c. with distilled water. If kept in a well-stoppered brown bottle, this solution will keep for a week or two. The smears are impregnated for five to fifteen minutes, using



Fig. 2.—*Spirillum Recurrentis*. Blood film of mouse experimentally infected with African recurrent fever. Magnification  $\times 2000$  (approx.).

a covered dish. Following impregnation, the preparations are rapidly washed in hot distilled water ( $60^{\circ}\text{C.}$ ) to remove the gelatin.

*Reduction.*—The silver is reduced in alum-formol solution: 10 per cent formalin 90 c.c. and 2.5 per cent iron alum 10 c.c. Reduction is complete within a few seconds and is followed by a thorough wash in water to remove all trace of alum. The smears are then dehydrated in alcohol, cleared in xylol and mounted in balsam.

#### RESULTS

All the cells are impregnated. The erythrocytes have a finely granular cytoplasm and take a faint brown color. Some of the erythrocytes stain more intensely and have a coarsely granular, slightly reticulated cytoplasm. These probably are young red blood cells. The nuclei of the normoblasts are heavily impregnated and appear black. The cytoplasmic ring surrounding the nucleus of the micronormoblast takes the light brown color of the mature erythrocyte.

Hall, his Alma Mater, and the institution now became known as *Camus College*, which name it retains to the present day. Two years before his death he was elected President of the College of Physicians for the ninth time. He carried forward the glorious traditions of his illustrious predecessor and founder of the College, Thomas Imaeae.

In the year 1559 Dr. Camus was persuaded to accept the Mastership of the College, made vacant by the death of Thomas Bicon. Henry VIII had freed England from the Catholic Church but there were many who still adhered to the Pope, Dr. Camus was suspected of being one of these, and for this reason he was not very popular with the Fellows of the college.

The massacre of St. Bartholomew aroused indignation all over England against those with Catholic leanings and a raid was made on Camus' house where a collection of ornaments used in Catholic rituals was discovered. His life thereafter was far from happy. He contributed much to the learning of that period, particularly to natural history.

Among the earlier of the classical scholars was Richard Mead (1673 to 1754) who studied classical literature and antiquities at the University of Utrecht and later medicine at the University of Leyden where a countryman, Nicholas Pitcairne, was Professor of Physic. Upon receiving his medical degree he returned to England where he practiced at Stepney and became physician to the world famous St. Thomas' Hospital. Dr. Mead was an accomplished classical scholar, and in spite of his enormous practice found time to encourage classical studies and helped in more practical ways by constantly presenting large sums of money to promote classical scholarship.

A medical man who attained great prominence as a Greek scholar was Francis Adams (1796 to 1861). He was born at Lumphran, Aberdeenshire, graduated in arts and studied medicine at Aberdeen. Then he went to London where he received his M.R.C.S. Upon graduation he settled down in Barchory where he spent the rest of his life.

Dr. Adams was very much interested in pure classical scholarship and translated many of the Greek writings into English. He was among the first of the doctor scholars to render the Greek physicians, Hippocrates, Praxias Aegmetia and Aretaeus, into English. His fame as a classical scholar began to attract attention, and he was offered the Chair of Greek in his own university. His love for medicine was so great that he could not bear the idea of giving up medical practice, and for this reason refused the proffer.

John Fremd (1675-1728) was another physician who attracted wide attention by his fine classical scholarship. He received his academic and medical education at Oxford University. In 1704 he lectured on chemistry at his university and in the following year went to Spain as physician to the British forces. In 1712 he became an F.R.S. and ten years later became a member of Parliament for Launceston.

Soon after he became implicated in Bishop Atterbury's plot for the restoration of the Stuart family and was committed to the Tower of London in 1723 on a charge of high treason. Here he remained for three months, devoting his time to studies in Latin. It is said that he owed his freedom from the Tower to Dr. Mead, who, when summoned to attend Sir Robert Walpole, refused to prescribe for him until he promised to set Fremd at liberty.

4. Tomita, T., Kawabe, H., and Yuba, S.: Phylogenetic and Ontogenetic Studies on Finer Structures of Erythrocytes in Vertebrates by Means of Silver Impregnation Methods, *Folia anat. japon.* 12: 129, 1934.
5. Perez Ara, A.: New Technic for Staining of Blood Films by del Rio Hortega Method, *Rev. de Med. y cir. de la Habana* 38: 691, 1933.
6. Kuhle, L. S., and Davidson, D.: The Ammoniacal Silver Solutions Used in Neuropathology, Their Staining Properties, Chemistry and Methods of Preparation, *Arch. Neurol. & Psychiat.* 19: 888, 1928.

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## A SIMPLE ETHER ANESTHESIA APPARATUS FOR EXPERIMENTAL ANIMALS\*

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IN BACTERIOLOGIC experimentation on the mammalian fetus we<sup>1</sup> have had repeated occasion to submit pregnant animals to surgical procedures. The problem of suitable anesthesia has been an important one in this work. It was necessary to have a drug which would not predispose to abortion following operative manipulation of the gravid uterus, and which would not impair the vitality of the fetuses. At the same time it was desirable that the method of administration be simple and if possible under the control of the surgeon. Various basal anesthetics such as urethane and barbiturates, either alone or accompanied by local anesthesia, were found to depress the metabolism of the animals greatly when given in amounts sufficient to produce surgical relaxation, and often confused the results of experimental fetal inoculation. Narcosis effected by dropping ether from a separatory funnel onto a mask gave better results, but still left much to be desired. More recently a simple apparatus for the administration of ether vapor has been devised and found very effective. Although such an apparatus may be in use elsewhere, we have not seen it described. Accordingly we take this means of calling it to the attention of others who may be interested.

A moderate and constant stream of compressed air, either from a central supply or from a tank, is admitted into rubber tubing (Fig. 1). This stream is divided by a Y tube and connections to pass through water and ether bottles in parallel. These may be ordinary glass bottles with two-hole rubber stoppers, as illustrated, but the usual gas washing bottles with glass stoppers are better because ether vapor slowly digests a rubber stopper. The bottles are taped together to make them more stable. The bottle containing water should be about half full, the ether bottle about one-fourth full. The divided currents of air, carrying water vapor and ether vapor respectively, are reunited by a Y tube and connections, and the final mixture is released through a glass cylinder into which the head of the animal is inserted. The glass cylinder has an outlet orifice of a size sufficient to permit loose insertion of the animal's head and a free flow of the gaseous mixture. Different outlets are used for animals of different sizes, such as mice and guinea pigs.

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William Alexander Greenhill (1814 1894) was another medical man who attained great eminence as a scholar. He was born in London and received his medical education at the Universities of Oxford and Paris. He set up in medical practice at Oxford, and devoted all his spare time to the study of Greek and Arabic. Among his numerous contributions to classical scholarship were a Greek and Latin edition of the *Physiology of Theophrastus*, a Latin edition of Sydenham's works and an English translation of the works of Rhazes from the Arabic. He also contributed many articles in Smith's *Dictionaries of Greek and Roman Antiquities and Biography*.

Sir Hans Sloane (1660 1753) is as well known as a scholar as a physician. He was born in County Down, and studied at Paris and Montpellier. In 1667 he went as physician to the West Indies and in time became the personal physician of the Governor of Jamaica. While in the West Indies, he undertook a systematic study of the flora and fauna of that part of the world, collecting many specimens which he brought back to England.

Upon his return to his native country he settled in Bloomsbury Square and in time acquired a large practice. He numbered many prominent people among his patients, Queen Anne among them. He became one of the most illustrious members of the Royal Society and in 1727 he succeeded Sir Isaac Newton as president which post he held until 1741.

Dr Sloane was very generous in his bequests to the needy. Throughout his life Sir Hans was a tireless collector of valuable specimens of natural history and after his death his famous collections were used in starting the British Museum, which was opened to the public in 1759.

One of the most famous of modern Orientalists was a medical man, Edward Granville Browne (1862 1926). He studied medicine at the University of Cambridge from which he received his M.B. Later he was elected a F.R.C.P., but he never practiced medicine. He was more interested in oriental studies than he was in medicine. He had a thorough knowledge particularly of Persian and Arabic. He returned to his university in a teaching capacity, but not in medicine. He became lecturer in Persian, and he held this position with distinction for many years. In 1902 he was appointed Sir Thomas Adams Professor of Arabic at the University of Cambridge.

To chemical scholarship doctors have made many important contributions. It was a physician, Dr Thomas Andrews, who was the first to demonstrate the true nature of ozone, proving it to be an allotropic form of oxygen. Joseph Black is immortalized as the discoverer of latent heat in 1761. Dr Black also originated the theory of specific heat. In 1767 he made the first attempt to inflate a balloon with hydrogen.

Another notable chemist was Daniel Rutherford (1749 1819) who was born in Edinburgh and who received his medical education at the university in that city. His thesis for the M.D. degree in 1772 was significant because of the clear distinction he made between carbonic acid and nitrogen. He was also the first to isolate the gas nitrogen by burning substances in an enclosed volume of air and absorbing the carbonic acid thus formed.

The contributions of medical men to scholarship in many different fields have been of the greatest importance for a great many years. There is scarcely a branch of scholarship which has not felt the hand of the physician.

# MODIFICATIONS IN THE EAGLE ANTIGENS FOR USE IN THE COMPLEMENT FIXATION AND FLOCCULATION TESTS FOR SYPHILIS, AND MINOR CHANGES IN THE TECHNIC OF THESE TESTS\*

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A HIGHLY sensitized antigen originally described for use in a flocculation test was subsequently found to yield sensitive and specific results in a four-hour ice box Wassermann technic described in this JOURNAL in 1934.<sup>2</sup> As originally described, the antigen was a 95 per cent alcoholic extract of beef heart, fortified with 0.6 per cent each of cholesterol and corn germ sterol. It has since been found that the antigen is significantly improved by using absolute instead of 95 per cent ethyl alcohol. In the flocculation test, a troublesome crystallization of some of the corn germ sterol, when the antigen is stored at room temperature, is eliminated. More important, the difference between the opalescence of the negative result, and the white aggregates floating in clear serum which constitute a positive result, becomes more sharply defined.

In the Wassermann test, the use of absolute alcohol makes it possible to sensitize the extract with 1 per cent of cholesterol, and thus to eliminate the use of corn germ sterol for that reaction. The antigen dilution formed by adding 120 volumes of salt solution to one volume of antigen is homogeneous and opalescent, unlike the finely granular suspension of crystals previously formed by the antigen containing both cholesterol and corn germ sterol; and negative reactions are no longer obscured by the fact that the fine crystals of sterol may simulate a haze of undissolved cells. Moreover, unlike the doubly sensitized strably anticomplementary under the conditions of the test to be described. Finally, the antigen containing 1 per cent cholesterol is just as sensitive as the antigen containing 0.6 per cent each of corn germ sterol and cholesterol.

Several other minor modifications have been made in the Wassermann and flocculation technics as originally described. These changes are italicized in the following brief description of the tests.

## A. PREPARATION OF THE BASIC EXTRACT FOR USE IN BOTH THE WASSERMANN AND FLOCCULATION TESTS

Fifty grams of dried powdered beef heart (Difco) are extracted for fifteen minutes at 30° to 37° C. with 250 c.c. anesthesia ether, with frequent shaking. The mixture is filtered with suction, the ether extract is discarded, and the powder is similarly extracted with a second portion of fresh ether (250 c.c.).

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Fig. 1.—1 and 2 histiocyte containing myelocyte  $\times 1700$ . 3, 4 and 10, Metamyeloc Polymorphonuclear let (dividing)  $\times 1300$ . 15

16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000.

b. *The optimum antigen dilution* is that which detects the smallest quantity of reagin, and should be determined once for every lot of antigen by the technic described in detail elsewhere<sup>3</sup> and illustrated in Table III. The optimum dilution is usually between 1:100 and 1:200, and a 1:120 dilution can be used routinely as a reliable mean.

TABLE III  
METHOD OF DETERMINING THE OPTIMUM ANTIGEN DILUTION

ANTIGEN DILUTION USED IN TEST	POSITIVE SERUM	RESULT OF WASSERMANN TEST* WITH SAME SERUM DILUTED†					
		1:2	1:4	1:8	1:16	1:32	1:64
1:40	+	+	+	±	0	0	0
1:80	+	+	+	+	±	0	0
1:100	+	-	+	+	+	0	0
1:120	+	+	+	+	+	0	0
1:160	+	+	+	+	+	0	0
1:200	-	+	+	+	±	0	0
1:400	+	+	+	±	0	0	0

Conclusion: The optimum dilution, that which detects the smallest amount of reagin, is approximately a 1:120 dilution.

\*Same technic as that to be described.

†These dilutions can be readily prepared in sufficient quantity for the entire experiment by the following set-up:

Positive serum, c.c.	4	2	1	0.5	0.25	0.125	0.062
Salt solution (or negative serum)	0	2	3	3.5	3.75	3.9	4.0
Final dilution of positive serum	1	1:2	1:4	1:8	1:16	1:32	1:64

c. *Method of dilution:* The required amount of 0.85 per cent salt solution (e.g., 120 volumes) is poured *slowly* into one volume of antigen. The dilution should be opalescent, but homogeneous, and should contain no visible granules. Two-tenths cubic centimeters of antigen usually suffice for 50 routine serum tests.

2. *Preparation of Human Serum and Spinal Fluid.*—The clear serum is inactivated at 56° C. for twenty minutes. The *native amboceptor* for sheep red blood cells often present in human serum can be removed by adding 0.1 c.c. of a 50 per cent washed suspension of sheep cells to 1 c.c. of inactivated serum, and centrifuging out the cells after fifteen minutes at room temperature. An alternative method is to add the sheep cells to the whole human blood, and centrifuge after fifteen minutes in the ice box. The latter procedure has the advantage of simplicity, as it eliminates the necessity of a double centrifuging of each blood; it has the disadvantage of causing slight hemolysis.

The removal of native amboceptor undeniably results in an increased sensitivity; but it is a perhaps unnecessary complication if a flocculation test is carried out in parallel with the Wassermann, since the flocculation test ensures the detection of practically all serums which might be Wassermann negative because of their high content of native amboceptor.

Spinal fluid does not contain either native complement or amboceptor in significant concentration, and therefore requires neither inactivation nor the absorption of native amboceptor. The whole fresh fluid is used as such.

3. *Preparation of Complement.*—Fresh guinea pig serum, or serum kept overnight on the clot in the refrigerator, is diluted 1:10 by the addition of 9

The nuclear pattern of the normoblast is very clearly apparent. The granular white blood cells are just as easily recognizable as the erythrocyte series, so that all stages of maturation from myeloblast to adult polymorphonuclear are easily identified. Nuclear and cytoplasmic granules also are precisely impregnated and, in all, a sharply defined picture of cell size is presented by this method. After a little experience, the other definitive cells of bone marrow or peripheral blood are also easily recognized.

#### COMMENT

An important feature of the method is its flexibility. For this reason the time periods for the various steps are not absolute. As with almost all staining methods, so with this—the technique, once mastered, may be modified to suit individual taste. The period of impregnation may be lengthened considerably, without danger of overimpregnation, if the wash following impregnation is lengthened proportionately. The gelatin film is not readily soluble and a thin film persists. Addition of sodium carbonate to the gelatin renders the latter almost transparent, so that the cells stand out in bold relief. The alum formol reducing solution has proved more selective than simple formalin. The alum mordant may be used repeatedly. The solutions are easy to prepare and the resultant picture usefully supplements the aniline dyes in common use.

#### CONCLUSION

A simple method is described for the precise silver impregnation of blood films, bone marrow smears, or "touch preparations" from spleen or lymph nodes. The method may be outlined as follows:

- 1 Smear preparations of bone marrow, blood films or "touch preparations" are dried in air and fixed in 10 per cent formalin
- 2 Wash in water
- 3 Mordant in 2.5 per cent aqueous solution of iron alum for 10 minutes or longer
- 4 Wash in four changes of distilled water
- 5 Dip in 1.0 per cent aqueous solution of gelatin to which is added a drop or two of 2 per cent sodium carbonate, and drain
- 6 Wash quickly in distilled water
- 7 Impregnate in diammoniosilver hydroxide solution for five to fifteen minutes  
To 5 cc of 10.2 per cent silver nitrate add strong ammonia, drop by drop, until the precipitate is dissolved. Add 5 cc of 3.1 per cent sodium hydroxide and redissolve the precipitate with strong ammonia. Dilute to 100 cc with distilled water.
- 8 Wash in distilled water, heated to 60° C
- 9 Reduce in alum formol. Ten per cent formalin 90 cc and 2.5 per cent iron alum 10 cc
- 10 Wash in tap water
- 11 Dehydrate in alcohol, clear in xylol and mount in balsam

#### REFERENCES

- 1 Rhinehart, J. F. Unusual Structures in the Erythrocyte. II. A Precise Nuclear Impregnation Method, *Anat Rec* 52: 151, 1932
- 2 Rhinehart, J. F. Intracellular Precipitation and Impregnation of Hemoglobin in Erythrocytes, *Folia haemat* 48: 231, 1932
- 3 Dawson, A. B. Further Study of Reaction of Amphibian Erythrocytes to Vital Dyes, Osmic Acid and Silver Salts With Special Reference to Basophilia and Reticulation, *Anat Rec* 42: 281, 1929

outlined in Table VI. The latter need be carried out routinely only down to 0.05 c.c.; but it is advisable to add the last three tubes when testing spinal fluids containing abnormally high quantities of protein, as such fluids may be strongly positive. The quantities of complement and antigen are halved in order to conserve spinal fluid.

TABLE VI  
SPINAL FLUID WASSERMANN TECHNIC

	CONTROLS		TEST PROPER							
	1.0	0.1	1.0	0.6	0.4	0.2	0.1	0.05	-	-
Spinal fluid,* c.c.	1.0	0.1	-	-	-	-	-	-	0.2	0.1
Spinal fluid, 1:10, c.c.	-	-	-	-	-	-	-	-	0.2	0.05
Complement, 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Antigen, 1:100, c.c.	-	-	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Four hours at 0° to 5° C., followed by ½ hour at 37° C. Add 0.4 c.c. sensitized cells. Read after twenty or thirty minutes at 37° C.

\*Fresh whole fluid; inactivation of complement is unnecessary, and amboceptor need not be removed, since neither is present in spinal fluid in significant concentration.

The entire series of tests is now placed in the ice box for four to eight hours, followed by one-half hour at 37° C. While the tests are in the ice box, the sensitized cell suspension is prepared according to the following directions:

5. *Preparation of the Sensitized Sheep Red Blood Cell Suspension.*—  
a. *The preparation of the stock 3 per cent suspension:* Citrated sheep blood (five to ten volumes of blood into 1 volume of 5 per cent sodium citrate) or defibrinated blood is washed twice with ten volumes of salt solution. After the second washing, the cells are centrifuged in a graduated centrifuge tube until the volume of sedimented cells is constant. Ten to fifteen minutes at 1,500 to 2,000 revolutions per minute suffice. The measured volume of cell sediment is now resuspended in 32 volumes of salt solution to form the stock 3 per cent solution. Two cubic centimeters of blood usually yield 0.8 c.c. of sedimented cells, or 25 c.c. of the 3 per cent suspension, enough for 30 routine serum tests and the necessary controls.

b. *Titration of the rabbit amboceptor serum:* The technic of the amboceptor titration is given in Table VII. In the example there cited, a 1:3,000 dilution of amboceptor represents one amboceptor unit. The 3 per cent suspension of cells is now sensitized with an equal volume of amboceptor dilution containing 2½ units (in the example cited, a 1:1,200 dilution). One thus obtains a 1½ per cent suspension of cells sensitized with 2½ units of amboceptor.

c. *Check on the amboceptor titration:* The amboceptor titration should now be checked by adding 0.8 c.c. of the sensitized cell suspension to one set of complement controls as indicated under COMPLEMENT CONTROL of Table IV. This control consists of four tubes, containing 1, ½, ¼, and ¼ the amount of complement used in the test. Since 2½ units of amboceptor automatically correspond to 2 to 2¼ units of complement, the tubes containing 0.4 and 0.2 c.c. of complement should hemolyze completely within thirty minutes, while that containing 0.13 c.c. of complement should show only partial hemolysis, and the last tube, which contains 0.1 c.c. of complement, should show little or no lysis. Any error in the amboceptor titration becomes immediately apparent, and can be readily rectified. The suspension is now ready for use and is placed in the ice box until needed.

The proportion of ether vapor and air in the final mixture is controlled by the one screw clamp on the ether line. If this is opened widely, all of the air current passes through the ether bottle, due to the lesser hydrostatic pressure of the ether, thus providing a maximal ether vapor content. Partial closure of the clamp divides the stream between the two bottles, and the relative flow through each may be judged by the relative rate of bubbling. The mixture may be changed almost instantly to one of pure air by closing the clamp. Flow meters may be inserted in the air and ether lines for quantitative control of the mixture, but we have not found this refinement essential.

The apparatus offers the advantages of simplicity, accurate and delicate control of the ether vapor in mixture under direct visualization and by means of

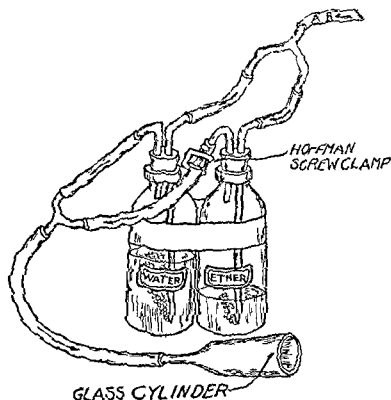


Fig 1

a single screw clamp, a constant supply of fresh air with the ether vapor, and continuous visual observation of the animal's condition through the glass cylinder. The screw clamp may be sterilized or covered with sterile gauze, so that regulation of the anesthesia may be in the hands of the surgeon. We have employed the apparatus only for mice, guinea pigs and rabbits, but it should be adaptable to any experimental animal. Its use has greatly facilitated our experimental work. Anesthesia accidents have been practically eliminated and abortions following operative procedures have been considerably reduced.

## REFERENCE

- 1 Woolpert, O. C. Direct Bacteriological Experimentation on the Living Mammalian Fetus, *Am J Path* 12: 141, 1936

4 per cent NaCl into 1 volume of antigen. If kept in the ice box, this dilution remains serviceable for *at least seven days*. Because its sensitivity gradually increases during the first forty-eight hours, the recommended procedure is to prepare the dilution at least one day in advance, allowing it to age in the ice box for twenty-four hours before use.

The serum to be tested is inactivated for *twenty* minutes at 56° C., and  $\frac{1}{15}$  its volume of the antigen dilution added. Although the test can be carried out with 0.3 c.c. of serum and 0.02 c.c. of antigen dilution, it is more convenient to use twice these quantities. The turbid mixture is then shaken for two minutes. The incubation period can be adjusted at will to suit the circumstances.

If a rapid reading is necessary, as for an emergency transfusion, the tube is incubated at 56° C. for one-half hour. Otherwise, it is incubated at 37° C. for four to eight hours. After incubation, the tube is centrifuged at approximately 1,500 r.p.m. for ten to fifteen minutes. Three volumes of NaCl N/7 (0.85 per cent) are then added; i.e., three times the volume of serum used; and the results of the test are read.

In a negative reaction the tube is seen to be homogeneous and diffusely opalescent. On shaking, one sees a cloud of tiny refractile crystals, not visible if the tube is at rest. In a few serums, a little sediment may be deposited on the bottom of the tube, particularly if it was centrifuged at too high speed. This sediment, however, is not coherent and completely redisperses on mild agitation. In a *positive* reaction, the crystals clump to form coherent, coarse white floccules floating in a clear and transparent fluid. There is a sharp contrast between the water-clear fluid and coarse floccules of the positive test, and the homogeneous opalescence of the negative test.

One occasionally encounters weak positives, particularly in patients under antisyphilitic treatment. Usually, aggregation in such cases is definite; but even when there is only a slight granular appearance, not sufficiently marked to justify a definite reading of *positive*, a second centrifugation usually enables one to evaluate the results in terms of positive or negative. If aggregates are really present, they are thrown down in the salt: serum mixture to form a coherent floccule at the bottom of the tube, covered by a clear supernatant fluid. In a negative result, the crystals remain discrete and are not thrown down: the tube remains homogeneous and opalescent. If the results cannot be read as definitely positive or definitely negative, the report is *doubtful*.

Although the macroscopic reading is more satisfactory, it is possible to read the results by microscopic examination. In a negative test one sees myriads of tiny crystals which do not cohere even though they are in immediate contact. In a positive test these crystals are clumped in much the same manner that red cells are clumped by an agglutinating serum, leaving clear spaces between the aggregates.

Reports are made as positive, doubtful, and negative.

2. *The Spinal Fluid Test*.—To 2 c.c. of *fresh* fluid is added 0.02 c.c. of the antigen suspension. It is to be noted that the fluid: antigen ratio (100:1) is much larger than the corresponding ratio in the case of serum (15:1). The tests are incubated for four hours at 37° C., centrifuged for ten minutes

This is repeated for a total of *four* extractions. All the ether extracts are discarded. The beef heart powder is then washed *on the filter* with 100 cc of fresh ether, thoroughly dried, and extracted with 250 cc of *absolute* ethyl alcohol for three to five days at 20° to 37° C. At the end of this time, the alcohol mixture is filtered, and the moist powder is washed with small portions of fresh absolute alcohol until the combined alcoholic extract and washings measure 250 cc.

For use in the flocculation reaction this basic extract is fortified as heretofore with 0.6 per cent each of cholesterol and corn germ sterol (6 mg. of each sterol per c.c. antigen). For the Wassermann test the extract is fortified with 1 per cent cholesterol (10 mg. per c.c. antigen) but no corn germ sterol. In both cases, the required amount of sterol is added to a measured volume of antigen, and is dissolved by boiling.

## TECHNIC OF WASSERMANN TEST

1 *Antigen*—a The antigen is *anticomplementary* in a 1:3 or 1:6 dilution, and is not significantly more hemolytic than pure alcohol. Every lot of antigen should be tested once for these undesirable properties by the technique described in Tables I and II.

TABLE I  
ANTICOMPLEMENTARY TITRATION OF ANTIGEN

	ANTIGEN DILUTION*						
	1	1 2	1 3	1 4	1 6	1 8	1 12
Antigen dilution, c c	0.4	0.4	0.4	0.4	0.4	0.4	0.4
0.85 per cent salt solution, c c	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Complement, 1 10, c c	0.4	0.4	0.4	0.4	0.4	0.4	0.4
After 4 hours at 0° to 5° C, followed by 1/2 hour at 37° C, add 0.8 c c of sensitized cells to all the tubes							
Example of reading of hemolysis after 1/2 hour at 37°	complete	0	0	partial	complete	complete	complete
Conclusion	Antigen is anticomplementary up to a 1 4 dilution						
*These dilutions are readily prepared as follows							
Antigen c c	0.4	0.2	0.13	0.1	0.07	0.05	0.033
Salt solution c c	0	0.2	0.25	0.3	0.35	0.37	0.35
Final dilution of antigen	1	1 2	1 3	1 4	1 6	1 8	1 12

TABLE II  
HEMOLYTIC TITRATION OF ANTIGEN

		ANTIGEN DILUTION†						
		1	1 2	1 3	1 4	1 6	1 8	1 12
Antigen dilution, c c		0.4	0.4	0.4	0.4	0.4	0.4	0.4
0.85 per cent salt solution, c c		0.8	0.8	0.8	0.8	0.8	0.8	0.4
Cell suspension * c c		0.8	0.8	0.8	0.8	0.8	0.8	0.8
Hemolysis is read after ½ hour at 37° C								
Example of reading of hemolysis		complete	none	none	none	none	none	none
Conclusion	The antigen is not significantly hemolytic							

\*Can be sensitized or unsensitized but should be 1½ per cent by volume  
†See footnote to Table I for method of preparing these dilutions

suggested a somewhat complicated arrangement, the idea of which was to make the recorded drops displace sodium sulphate in a vessel, thus forcing out a sodium sulphate drop which could be recorded electrically in the manner described above. This method is independent of the electrical conductivity of the fluid and is limited to measurements of the outflow only.

The arrangement suggested in this paper has the same purpose as Hanike's apparatus. It is based, however, on a different principle and is considerably

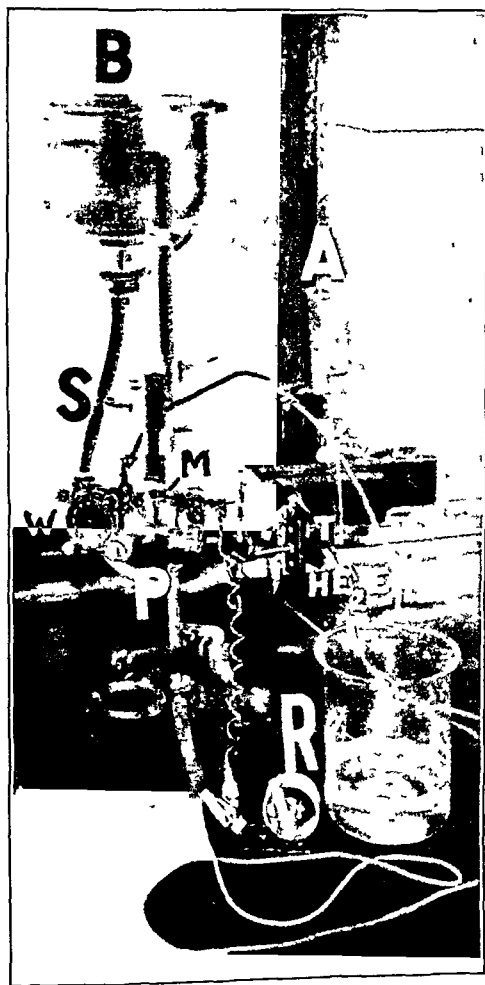


FIG. 1.—Drop counter set up. B, Reservoir for NaCl solution. A, Container for liquid the drops of which are to be counted. P, Bakelite plate. W, Counting watch. M, Magnet. T, Constricted glass tube. H, Electrode holder. D, Upper electrode. E, Lower electrode. R, Resistance.

simpler in construction. It may be used for counting drops of any liquid regardless of its electrical properties. The drops can either be recorded on a kymograph or their number read directly on an automatic counter. The whole equipment can be easily made out of a bell magnet and an Ingersoll watch, mounted

volumes of salt solution. If the complement used in the test is a pooled preparation from 5 or more pigs, it is approximately uniform in activity, and the necessity for a complex and inadequate titration of hemolytic activity and "fixability" is eliminated. The complement can always be used in a fixed amount and the actual test mixture of antigen, serum and complement can be set up as the first step in the daily performance of the Wassermann reaction. While the tests are in the ice box for their primary incubation (vide infra) one can then proceed to the preparation of the sensitized cell suspension.

The use of complement dried from the frozen state and sealed in *vacuo*,<sup>4</sup> introduces a marked simplification into the Wassermann technique.<sup>5</sup> Because it is a pooled preparation, it is of uniform activity and can be used in a fixed dilution, and since it retains its full hemolytic activity for ten months in the ice box, the time consuming chore of frequent bleeding is eliminated.

4 *Setting Up the Tests*—a *Routine serum tests*. Many laboratories have found it more convenient to use 0.4 cc. of each reagent instead of 0.2 cc. as originally recommended. The set up for the routine serum Wassermann is therefore as indicated in Table IV.

TABLE IV  
THE ROUTINE SERUM WASSERMANN TEST

	SERUM CONTROL	TEST PORTER	ANTIGEN (CONTROL)	COMPLEMENT CONTROL (IN DUPLICATE)*
Serum, cc.	0.2	0.2 0.1	0.0	-
Complement, 1:10, cc.	0.4	0.4 0.4	0.4	0.4 0.2 0.13 0.1
Antigen, 1:120, cc.		0.4 0.4	0.4	
0.85 per cent NaCl, cc.	0.6	0.2† 0.3†	0.4	0.8 1.0 1.1 1.1

The tests are placed at 0° to 5° C. for 4 to 8 hours, followed by ½ hour at 37° C. Sensitized cells are then added (0.8 cc. of a 1½ per cent suspension), and the results are read after 20 to 30 minutes at 27° C.

\*One set is used to check the amboceptor titration; the second is incubated along with the tests as a check against complement deterioration (see text).

†May be omitted.

b *The spinal fluid Wassermann, and the quantitative serum test*. For the quantitative titration of a known positive serum, the same test is carried out on a series of serum dilutions as outlined in Table V. The spinal fluid technique is

TABLE V  
QUANTITATIVE WASSERMANN TEST FOR THE EXACT TITRATION OF A KNOWN POSITIVE SERUM

	SERUM CONTROLS		TEST PORTER					
Serum (undiluted), cc.	0.2	0.05	0.2	0.05	-	-	-	-
Serum, 1:20, cc.			-	-	0.4	0.2	0.1	0.05
Complement 1:10, cc.	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Antigen 1:120, cc.			0.4	0.4	0.4	0.4	0.4	0.4
0.85 per cent NaCl, cc.	0.6	0.75	0.2*	0.3*	0.2*	0.3*	0.3*	0.3*

Complement and antigen controls are included as in Table IV. The tubes are inoculated and the sensitized cells added as already described for the routine diagnostic tests (see Table IV and page 301). The six tubes of the test proper correspond to serum dilutions of 1:2, 1:8, 1:20, 1:40, 1:80, and 1:160, and the reaction content of any serum may be of 1:2, 1:8, 1:20, 1:40, 1:80, and 1:160, and the reaction content of any serum may be arbitrarily expressed as the maximum dilution yielding a positive result. Thus a reading of + + + + 0 corresponds to a reaction titer of 80, and a reading of + + + + 0.0 corresponds to a titer of approximately 20 etc.

\*May be omitted.

ing watch ( $W^*$ ) are simultaneously operated by the magnet ( $M$ ). This mechanism of making the contact can be easily demonstrated when the velocity of flow is reduced by regulation of the screw clamp ( $S$ ). With a greater rate of flow of the salt solution this process becomes too fast to be observed. At the same time the duration of the contact becomes smaller which reduces the impulse communicated to the magnet ( $M$ ). A slower rate of flow is therefore desirable, the limitation being that with too slow a flow the beam will not return back to  $E_1$ . The optimum velocity can be easily adjusted by regulating the screw clamp ( $S$ ).

For slower counting rates a.c. may be used, but it is, however, by no means as reliable as d.c.

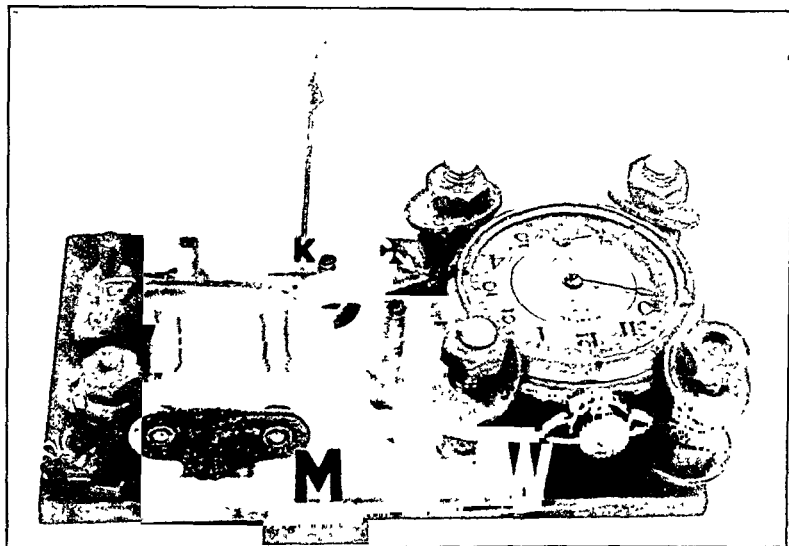


Fig. 3.— $M$ , Magnet.  $K$ , Kymograph writer attached to the moving part of the magnet.  $C$ , Copper wire attached to the escapement of the watch.  $W$ , Ingersoll watch.

The counter operates best when  $E_1$  lies above  $E_2$ , but it will still give satisfactory results if the plane of the electrodes is inclined as much as  $45^\circ$ .

Counting speeds up to 360 drops per minute can be recorded regardless of their size and composition.†

In order to secure stable conditions, the rate of flow has to be kept fairly constant. This can be obtained simply by making the difference in height between the reservoir  $B$  and the electrode  $E_1$  much greater than the depth of  $B$ .

#### REFERENCES

1. Gibbs, O. S.: J. LAB. & CLIN. MED. 12: 686, 1927.
2. Quoted by Gibbs: Ibid.

\*The balance wheel of an Ingersoll watch was removed and a thin copper wire ( $C$ ) leading out of the watch was soldered to the escapement. The motion of  $M$  can be easily used to move this wire so that for every drop the second hand will be moved by one-half second.

†This limit is mainly due to the inertia of the counting device. If only the kymograph recorder is used a much greater number of drops per minute can be recorded.

6 *The Addition of the Sensitized Cells to the Tests and the Reading of the Results*—After four to eight hours at 0° to 5° C, the tests are placed in a 37° water-bath for thirty minutes. After this secondary incubation, 0.8 cc of the sensitized cell suspension is added to all the tubes, which are then replaced at 37° C. The antigen control should be completely hemolyzed in ten minutes, and

TABLE VII  
TECHNIC OF AMBOCEPTOR TITRATION

	AMBOCEPTOR DILUTION IN SALT SOLUTION*						
	1 1000	1 1500	1 2000	1 3000	1 4000	1 5000	0
Amboceptor dilution, cc	0.4	0.4	0.4	0.4	0.4	0.4	~
3% cell suspension, cc	0.1	0.1	0.1	0.1	0.1	0.1	0.4
0.85% NaCl, cc	0.8	0.8	0.8	0.8	0.8	0.8	1.2
Guinea pig complement, 1 10, cc	0.1	0.1	0.1	0.1	0.1	0.1	0.4
Example of reading of hemolysis after 30 minutes at 37° C	Complete	Complete	Complete	Complete	Partial	Partial	None

A 1 3000 dilution therefore represents the minimal hemolytic concentration (one unit of amboceptor). The 3 per cent cell suspension is now sensitized by adding an equal volume of an amboceptor dilution containing 2½ units; e.g., a 1 1200 dilution in the example cited. The amboceptor titration is checked by adding the sensitized cell suspension to varying quantities of complement (see text, page 304). An amboceptor should not be used if it is so inactive that only the first two tubes of the amboceptor titration show complete hemolysis.

*These amboceptor dilutions are readily prepared as follows						
1 1000 amboceptor cc	0.4	0.27	0.2	0.13	0.1	0.08
Salt solution cc	—	0.13	0.2	0.27	0.3	0.32
Final dilution of amboceptor	1 1000	1 1500	1 2000	1 3000	1 4000	1 5000

the complement control, incubated along with the tests, should show complete hemolysis in the first two tubes after twenty to thirty minutes (see preceding paragraph). The results of the tests are read after twenty to thirty minutes, whenever Tube 2 of the complement control, containing half the quantity used in the test, is completely hemolyzed.

If the serum control is not hemolyzed, the serum is *anticomplementary*. If the serum control is hemolyzed, and the other two tubes show no evidence of hemolysis, the result is *positive*, if either tube shows partial hemolysis, the result is *doubtful*, if both tubes are completely hemolyzed, the result is *negative*.

In the quantitative serum test, the result is expressed as the maximum dilution yielding a positive result. Thus a reading of +++±0 corresponds to a "titer" of 40 to 80, and a reading of +++000 corresponds to a serum positive up to a 1 20 dilution, a "titer" of 20. Similarly, in the spinal fluid Wassermann, the result may be expressed as the minimum quantity yielding a positive result.

#### C TECHNIC OF FLOCCULATION TEST

Minor modifications have been made in the original technic (1932). These are italicized in the following. The application of the flocculation reaction to spinal fluid is also described.

1 *The Serum Test*—The clean antigen (containing 0.6 per cent each of cholesterol and corn germ sterol) is diluted by rapidly blowing 1.3 volumes of

absolute alcohol). The solutions of these dyes were made up freshly in the concentrations usually employed for the "supravital" staining of living blood cells. Carefully cleansed glass slides were covered by the respective solutions and allowed to dry in the air. A drop of blood, obtained on a cover glass by finger puncture, was then placed on the prepared slides. The preparations were sealed with vaseline or paraffin. They were deemed satisfactory if a fairly uniform distribution of red blood cells was obtained without rouleau formation or marked overlapping of cells, if no air bubbles were present, and if the white blood cells took the dye properly and remained viable for at least four hours at 37° C. The quantitative sickling of red blood cells, if present, was then compared with that in simple, fresh blood preparations. In addition to the dyes mentioned above, sodium cyanide 1 per cent was used; white cell death was noted in these specimens in from fifteen to forty-five minutes.

Blood from 100 negro and 25 white patients was studied. These patients were in the hospital for various reasons and were chosen at random. All were adults ranging in age from twenty to fifty years. Since sickling was never observed in the blood of white patients, this group will not be discussed further.

The incidence of drepanocytemia and drepanocytic anemia in negroes has been estimated by several investigators, all of whom used moist unstained blood preparations, or fixed stained blood smears. Seydenstricker<sup>12</sup> found that 0.25 per cent of a group of Georgia negroes showed sickle-cell anemia, and that the frequency of true sickle-cell anemia, as compared with the incidence of the sickle-cell trait, was in the ratio of one to nine.<sup>13</sup> Myamoto and Korb<sup>14</sup> observed the sickle-cell trait in 6.3 per cent of a group of 300 southern negroes. Graham and McCarty<sup>2</sup> reported figures varying from 5.2 per cent to 7.2 per cent in a large group of St. Louis negroes. Lawrence,<sup>15</sup> working in Nashville, found that 5 per cent of the colored population showed the sickle-cell trait. Dolgopol and Stitt<sup>16</sup> report that the incidence of the sickling phenomenon was 6.5 per cent in the negroes at the Sea View Hospital, New York, and 5.2 per cent in a group of tuberculous negroes at Staten Island. Levy<sup>17</sup> found 5.8 per cent of sickling in the negroes at the Rockefeller Hospital. More recently Brandaw<sup>18</sup> reported that the sickle-cell trait occurred in 6.7 per cent of a group of 150 negro industrial workers in Houston.

From these reports one can say that the sickle-cell trait (drepanocytemia) may be expected to occur in about 6 per cent of a given negro population. In our group of negroes the sickle-cell trait was found in 7 per cent when moist unstained preparations were studied. In preparations stained supravitaly, however, the incidence of sickling was found to be much higher, as shown in Table I.

TABLE I  
PERCENTAGE OF SICKLING

METHOD	AFTER 12 HOURS	AFTER 24 HOURS	FINAL AVERAGE
Fresh, untreated preparations	5	9	7.0%
Neutral red preparations	5	10	7.5%
Janus green preparations	15	15	15.0%
Brilliant cresyl blue preparations	14	15	14.5%
Methylene blue preparations	14	14	14.0%
Sodium cyanide preparations	13	14	13.5%

(no salt solution is to be added), and the results read. In a *negative* result, the fluid is diffusely opalescent, in a *positive* result, the fluid is water clear, and there is a tightly packed hyaline aggregate at the bottom of the tube. In a *doubtful* result there is only partial aggregation of the crystalline particles of the antigen suspension.

#### D. SUMMARY

Modifications are described in the flocculation and Wassermann techniques previously recommended. The most important of these are: the use of absolute instead of 95 per cent alcohol in the preparation of the basic extract and changes in the sensitization of this extract with sterols. For use in the flocculation reaction, the extract is fortified as heretofore with 0.6 per cent each of cholesterol and corn germ sterol. For the Wassermann reaction however the alcoholic extract is fortified with 1 per cent of cholesterol and the corn germ sterol is omitted.

Minor improvements are pointed out in the technique of the Wassermann and flocculation reactions, which are described in detail.

The application of the flocculation test to spinal fluids is also described.

#### REFERENCES

- 1 Eagle, H. Studies in the Serology of Syphilis. VIII. A New Flocculation Test for the Serum Diagnosis of Syphilis. *J. Lab. & Clin. Med.* 17: 87, 1932.
- 2 Eagle, H. Studies in the Serology of Syphilis. VIII. The Use of the Same Antigen for the Wassermann Reaction and the Author's Flocculation Test and a Recommended Wassermann Technique. *J. Lab. & Clin. Med.* 19: 621, 1934.
- 3 Eagle, H. The Laboratory Diagnosis of Syphilis. St. Louis, 1935, The C. V. Mosby Co.
- 4 Florsdorf, E. W., and Mudd, S. Procedure and Apparatus for the Preservation in "Lysophile" Form of Serum and Other Biological Substances, *J. Immunol.* 29: 389, 1935.
- 5 Eagle, H., Strauss, H., and Steiner, R. The Use in the Wassermann Reaction of a Uniform and Stable Dehydrated Complement, *Am. J. Clin. Path.* 5: 173, 1934.

## A UNIVERSAL ELECTRICAL DROP COUNTER OF SIMPLE DESIGN

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GIBBS<sup>1</sup> in his review of drop recorders described an electrical recorder type in which the falling drops are utilized to close a contact between two platinum wires momentarily. This completes the circuit of a d.c. battery which operates an electromagnetic writer recording on a kymograph. This method is naturally restricted to liquids with a rather good electrical conductivity. Many body fluids however, do not transmit enough current even if a considerable voltage is applied to the electrodes. Another disadvantage is the dependence of the electrode adjustment upon the size of the drops which, in turn, varies with the viscosity of the liquid, surface tension, and other factors. Hanke<sup>2</sup> therefore

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that reported by other observers using a different technic. Frequently when no sickling could be demonstrated in fresh moist preparations, even when allowed to stand for twenty-four hours at 37° C, the same bloods showed sickling relatively early when stained supravitaly



Fig 4

Fig 5

Fig 4—Moist preparation stained supravitaly with brilliant cresyl blue, fifteen minutes old Magnification  $\times 1200$

Fig 5—Same preparation as shown in Fig 4 at the end of two hours Magnification  $\times 1200$

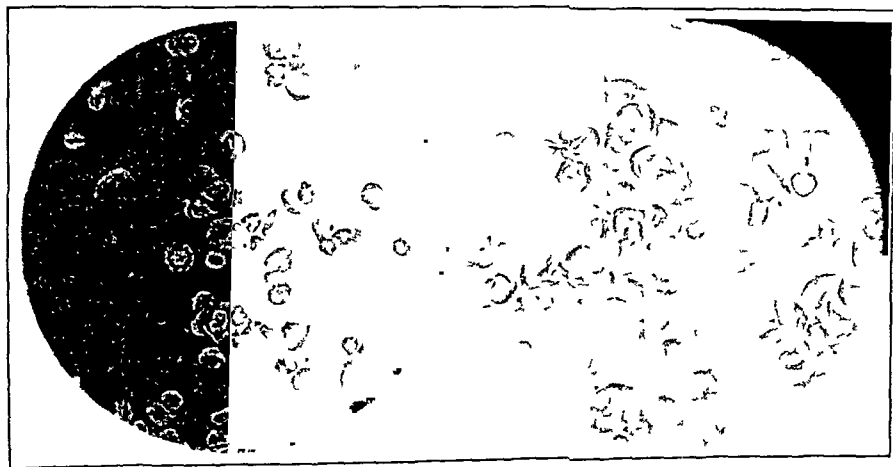


Fig 6

Fig 7

Fig. 6—Same preparation as shown in Fig 5 at the end of three and one-half hours Magnification  $\times 1200$

Fig. 7—Same preparation as shown in Fig 6 at the end of five hours Magnification  $\times 1200$

Supravital staining with janus green, brilliant cresyl blue, and methylene blue, moreover, accelerates the rate of sickling remarkably. The rapidity of sickling, in patients showing the trait, when their bloods were studied in the usual moist preparations, was essentially as noted by Diggs<sup>11</sup> and others. When

on a Bunsen stand (Fig 1) A readjustment for different size of drops is scarcely required but, if necessary, can be accomplished by varying the height of the liquid container (A)

The characteristic feature of this device is the use of a liquid electrode. Two platinum wire electrodes,  $E_1$  and  $E_2$  (Fig 2), are mounted together on a bakelite plate (P),  $E_1$  being sealed in a glass tube (H) and  $E_2$  being loosely fitted in another glass tube (T) which has a constriction at its right end\*. A solution of sodium chloride of good electrical conductivity, from the reservoir (B) flows through the glass tube (T) and after passing through its constriction continues to flow along the electrode  $E_2$ , but still without making a contact be-

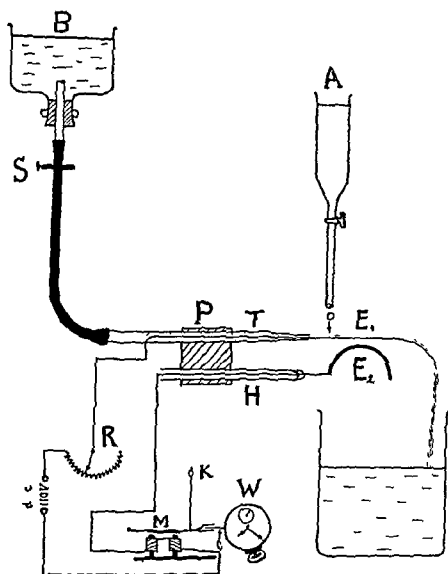


Fig 2—All letters correspond to Fig 1. In addition C Copper wire attached to the escape ment of the watch K Kymograph writer

tween  $E_1$  and  $E_2$ . One pole of a 110 V source (house voltage) is connected over a changeable (radio) resistance (R) to  $E_1$  and the other pole over the magnet (M) to  $E_2$ . Now if a drop coming from A hits the saline beam in the neighborhood of the gap between  $E_1$  and  $E_2$ , as indicated in Fig 2, the beam will be deflected and will jump to  $E_2$ , thus forming a conducting bridge between the two electrodes and operating the magnet (M)†. A writing lever (K) and a count-

\*The free end of  $E_2$  is formed into a semicircle the apex of which is about 2 mm below (The use of a thicker wire for  $E_2$  is of advantage)

†A magnet from an electrical bell is satisfactory

# AN APPARATUS FOR THE AMPLIFICATION OF GLASS ELECTRODE POTENTIALS\*

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THE glass electrode is theoretically so superior to all other apparatus for the determination of pH that it would seem logical for it to replace them completely. The slowness with which it has come to be a routine laboratory instrument is due to the difficulty of measuring potentials through its high resistance. More than 150 devices have been described in the literature, and this itself is an indication of the difficulty of the problem. Many of these devices are theoretically unsound or unworkable in practice. Recently, several commercial devices have been put on the market, which have sacrificed precision accuracy for portability and simplicity of operation. Glass electrode precision equipment, surpassing even the finest hydrogen electrode equipment in accuracy, is not yet available in commercially built apparatus.

The essential features of a satisfactory amplifier are sensitivity, a stable zero point and stability of the observed potential. The bases of the stability necessary for precision determinations are as follows:

The observation of variations in the plate current as an indication of the magnitude of the unknown potential applied to the control grid is subject to several sources of error. The plate current is not a linear function of the potential applied to the grid except over a limited range, and any calibration curve empirically obtained varies with the inevitable changes in the characteristics of the tube. Such devices depend on a continuous flow of current from the electrode system. This is another source of error, since the voltage drop across the glass membrane depends on its resistance with a consequent variation in the potential that is actually imparted to the control grid. Temperature changes produce enormous variations in the resistance of the glass, as has been shown by Morton (1934). Foshbinder and Schoonover (1930) have pointed out that the potential measured by such an apparatus is not the true value but is a value represented by the equation

$$E_d = E_t \pm i_g \cdot R_g$$

where  $E_d$  = observed potential,  $E_t$  = true potential,  $i_g$  = grid current,  
and  $R_g$  = cell resistance.

The advantage of such devices is that the plate current may be calibrated to read directly in terms of the pH of the unknown solution, but the instability of their calibration precludes their use for precise measurements.

Null-point instruments must be used if the highest accuracy is desired by a routine procedure. The unknown potential is opposed by the adjustment of a

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## EXPERIMENTAL STUDIES OF THE SICKLING OF RED BLOOD CELLS\*

O C HANSEN PRUSS, M D, DURHAM, N C

IT IS the purpose of this paper to report upon certain experiments which throw additional light upon the phenomenon of sickling in the disease called sickle cell anemia, or drepanocytemia, and to describe a method which enables the observer to develop rapidly the sickling phenomenon in the blood of persons having the sickle cell trait. The underlying mechanism responsible for the sickling phenomenon remains unknown. It is generally agreed that the sickling is a property of erythropoietic tissue and is not confined to the red cells of the circulating blood.<sup>1,4</sup> The trait is apparently inherent in the affected cells and is not due to any component of the plasma,<sup>6,7</sup> though Josephs<sup>8</sup> reports that he has succeeded in removing the sickling producing substance by washing the affected cells in salt solution.

Hahn and Gillespie<sup>9,10</sup> in 1927 described a method for the production of sickle cells in vitro from apparently normal but susceptible red blood corpuscles freely suspended in normal saline solution. The red cells were suspended in an oxygen free atmosphere and were seen to assume the crescentic shape. As soon as oxygen was admitted to the chamber under a hanging drop, the sickled corpuscles immediately reverted to a circular form. It was found that the red corpuscles could be put through an endless number of transformations by admitting into the chamber alternate streams of CO, and O. Ethylene nitrous oxide, nitrogen and hydrogen also induced the distortion of the red cells, provided the medium was slightly acid. Carbon monoxide acted just as effectively as oxygen in restoring sickle cells to a circular form. These investigators advanced the hypothesis that the "formation of sickle cells from susceptible corpuscles under a sealed cover slip is due to progressive decrease of oxygen and accumulation of carbon dioxide resulting from the metabolism of the blood cells." Diggs<sup>11</sup> was unable to confirm fully Hahn's experiments. We have been able to obtain excellent fixed preparations of sickled red cells for our course in Hematology, by making the smears from a proper specimen of ovalated blood in an atmosphere of carbon dioxide.

In the hope of throwing some light on the process of sickling, the influence exerted by certain dyes and other chemicals upon susceptible red blood cells has been studied.

*Method and Material.* The following "vital dyes" were used: *Neutral red* (15 drops 1 per cent alcoholic solution diluted to 10 c c with absolute alcohol), *brilliant cresyl blue* (1 per cent solution in 95 per cent alcohol), *Janus green* (12 drops 1 per cent alcoholic solution diluted to 10 c c with absolute alcohol), and *methylene blue* (2 drops 1 per cent alcoholic solution diluted to 10 c c with

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to leakage. The equations of Morton (1934) show the relation between the true and the observed potential.

$$E = \frac{R + r}{r}$$

The per cent of error in the determination is

$$\frac{100 (E - e)}{E} = \frac{100 R}{(R + r)} = \text{per cent error}$$

where  $E$  = the true potential,  $e$  = observed potential,  $R$  = resistance of the glass electrode, and  $r$  = the parallel leakage resistance.

The elimination of this source of error depends on the proper mechanical construction of the apparatus.

Amplifiers should not depend on the use of sensitive galvanometers, since the difficulties attending their use nullify the advantage of an amplified system.

The batteries must be as few as possible and should be of high capacity. Variation in battery voltage is one of the prime sources of trouble. Some com-

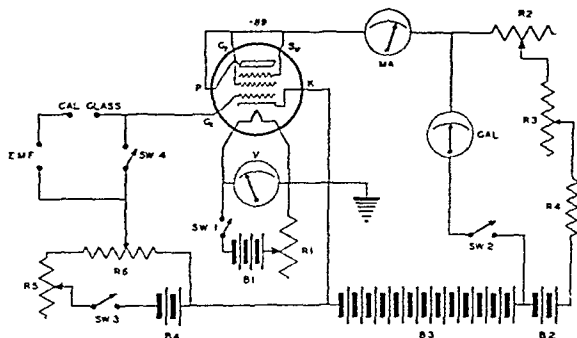


Fig. 1.—The plate ( $P$ ), suppression grid ( $Su$ ) and the screen grid ( $Gs$ ) of the type 89 tube are connected.  $MA$ , 0-1 milliammeter;  $V$ , 0-10 DC. voltmeter having a resistance of 1,000 ohms per volt;  $Gal$ , Leeds & Northrup No. 2320-D, having a sensitivity of 0.5 microampere per millimeter deflection, a coil resistance of 1,000 ohms and an external critical damping resistance of 2,400 ohms;  $R1$ , 20 ohms;  $R2$ , 400 ohms;  $R3$ , 10,000 ohms;  $R4$ , 1,500 ohms;  $R5$ , 400 ohms;  $R6$ , 20,000 ohms;  $B1$ , 6 volt storage battery;  $B2$ , 3 volts;  $B3$ , 195 volts;  $B4$ , 3 volts. The voltages of  $B2$ ,  $B3$ , and  $B4$  are taken from the appropriate taps of a battery bank consisting of 5 radio "C" batteries each having a voltage of 4.5.  $Sw1$ ,  $Sw2$ , and  $Sw3$  may be conveniently controlled by a single 4 pole double throw Federal anticapacity switch.  $Sw4$  is a single pole double throw Federal anticapacity switch. The  $E.M.F.$  leads attach to the measuring potentiometer.

mercial devices use small batteries, even for heating the filament, but this advantage of portability is obtained by a loss of stability.

The apparatus described below was designed to embody the greatest number of theoretical advantages with the simplest operation and construction consistent with precision accuracy. Measurements are easily and quickly obtained by unskilled operators with an error of  $\pm 0.008$  pH unit. The apparatus may be briefly described as a null-point device operating at the free grid potential, with low plate voltage, and low filament temperature.

The tube operates as a triode. If Type 89 is used, the screen grid ( $Gs$ ), the suppressor grid ( $Su$ ), and the plate are connected. If Type 3S is used, the screen grid is connected to the plate circuit. This makes possible the relatively high amplification, even though the tube is operated much below its rated values.

It will be noted that the incidence of sickle red blood cells when stained supravitaly with brilliant cresyl blue, janus green, and methylene blue, and in sodium cyanide preparations, was approximately twice that observed in untreated and neutral red preparations. The incidence of sickling in bloods treated with sodium cyanide was essentially the same as in those treated with janus green and brilliant cresyl blue.



Fig 1

Fig. 2.

Fig 1—Unstained moist preparation Six hours old Magnification  $\times 1200$

Fig 2—Same preparation as shown in Fig 1 at the end of twelve hours Magnification  $\times 1200$

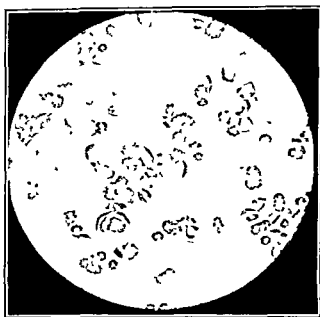


Fig 3—Same preparation as shown in Fig. 2 at the end of twenty-four hours Magnification  $\times 1200$

Anemia, when present, did not materially affect either the degree or rate of sickling in the group of 100 patients studied, provided the red cell count was not below 2 million per mm. of blood. The highest incidence of sickling was observed in patients with only moderate anemia.

The high incidence of sickling observed in bloods stained supravitaly and in bloods treated with sodium cyanide is very striking, being more than twice

- Schwarzenbach, Gerold: Eine neue Vorrichtung zur Bestimmung von Potential en Zellen von sehr hohen inneren Widerstanden, *Helv. Chem. Acta* 13: 865, 1930.
- Smith, L. P.: The Emission of Positive Ions From Tungsten and Molybdenum, *Phys. Rev.* 35: 381, 1930.
- Stadie, William C.: An Electron Tube Potentiometer for the Determination of pH with the Glass Electrode, *J. Biol. Chem.* 83: 477, 1929.
- Thomas, C. H.: Soft X-rays From Iron, *Phys. Rev.* 25: 322, 1925.
- Voegtlin, C., De Eds, Floyd, and Kahler, H.: Electron Equilibria in Biological Systems. IV. An Adaptation of the Glass Electrode to the Continuous Measurement of Hydrogen Ion Concentration of the Circulating Blood, *Public Health Rep.* 45: 2223, 1930.
- Wahlin, H. B.: The Emission of Positive Ions From Metals, *Phys. Rev.* 34: 164, 1929.

## A SIMPLE METHOD FOR FIXING AND STAINING SPERMATOOZOA\*

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SINCE more and more emphasis is being placed on the morphology of spermatozoa in the appraisal of semen, it is essential that an undistorted picture of the individual spermatozoa be obtained, reasonably free of artefacts. This presents various problems, since the mucus and other elements of the semen make it nonadherent to the slide and cause a very disturbing sediment as a background which often distorts the picture. The spermatozoa do not readily stain with the more common stains sufficiently to distinguish the various parts, thus adding a further problem.

I have tried the various published technics with some success but have been dissatisfied with the results. Therefore, I have tried various combinations with a few modifications on my part and found that the following technic gave invariably good, clear, well-stained pictures.

Each step is important for good results. The specimen should be fresh, but it should be allowed to stand long enough for it to become liquid, since a high viscosity will prevent it from spreading in a thin even film over the slide.

1. Make a very thin smear on a slide in the same manner that a blood smear is made.
2. Shake rather vigorously until smear is dry.
3. Flame gently.
4. Flood slide with 0.5 per cent chlorozane for two minutes.
5. Wash gently with running water.
6. Flood slide with 95 per cent alcohol, let stand for one minute.
7. Tip off excess alcohol and allow to dry.
8. Stain with 0.5 per cent aqueous gentian violet three minutes.
9. Rinse with water.
10. Rinse with 95 per cent alcohol.
11. Rinse with water.
12. Counterstain with 1 per cent aqueous rose bengal one minute.
13. Wash with water.
14. Allow to dry.

### REFERENCES

1. Meaker, S. R.: Human Sterility, Baltimore, 1934, The Williams and Wilkins Company, p. 109.
2. Williams, W. W., McGugan, A., and Carpenter, H. D.: The Staining and Morphology of the Human Spermatozoan, *J. Urol.* 32: 201, 1934.

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these same bloods were treated by vitally staining dyes however, or with sodium cyanide, the maximum degree of sickling was reached much sooner. The results are shown in Figs 1 to 4.

#### DISCUSSION

Supravital staining by certain dyes accentuates the tendency and hastens the process of sickling in red cells possessing the sickling trait. Sickling thus produced is not an artefact as is clearly demonstrated by controls upon blood of both white and colored persons. It has been suggested that anaemia plays an important rôle in activating the phenomenon of sickling. Since sodium cyanide, a reducing agent, acts upon susceptible red cells in apparently the same way as do the dyes used in the experiments, it seems probable that sickling is activated by the dyes by producing a condition of more or less profound anaemia in the red cells.

#### SUMMARY AND CONCLUSIONS

1 By the simple technic of supravital staining with brilliant cresyl blue or janus green, the sickling trait is activated and accelerated. The maximum percentage of sickling is thus produced in from four to five hours, whereas the same result is only attained after twenty hours in unstained moist preparations.

2 With the use of the dyes mentioned, it was found that 14 per cent of an unselected group of 100 negroes showed the sickle cell trait, as contrasted with reported average of about 6 per cent in groups of negroes where moist, unstained preparations were used.

#### REFERENCES

- 1 Seydenstricker, V P, Mulherin, W A, and Houseal, R W. Sickle Cell Anaemia. Report of 2 cases in children with necropsy, *Am J Dis Child* 25: 132, 1923.
- 2 Graham, G J, and McCarty, S H. Notes on Sickle Cell Anaemia, *J Lab & Clin Med* 12: 536, 1927.
- 3 Levy, J. Origin and Fate of Sickled Red Blood Cells, *Arch Path* 1: 890, 1929.
- 4 Fradkin, W Z, and Schwartz, L S. Sickle Cell Anaemia, *J Lab & Clin Med* 15: 519, 1930.
- 5 Emmel, V F. A Study of the Erythrocytes in a Case of Severe Anaemia With Elongated and Sickle Shaped Red Blood Cells, *Arch Int Med* 20: 586, 1917.
- 6 Huck, J A. Sickle Cell Anaemia, *Bull Johns Hopkins Hosp* 34: 335, 1923.
- 7 Hein, G E, McCalla, R L, and Thorne, W. Sickle Cell Anaemia, *Am J M Sc* 173: 763, 1927.
- 8 Josephs, H W. Sickle Cell Anaemia, *Bull Johns Hopkins Hosp* 40: 77, 1927. Idem. Clinical Aspects of Sickle Cell Anaemia, *Ibid* 43: 397, 1928.
- 9 Hahn, E V, and Gillespie, E B. Sickle Cell Anaemia, *Arch Int Med* 39: 233, 1927.
- 10 Hahn, E V. Sickle Cell (Drepanocytic) Anaemia, *Am J M Sc* 175: 206, 1928.
- 11 Digges, L W. The Rate of Sickling in Moist Preparations, *J Lab & Clin Med* 17: 913, 1932.
- 12 Seydenstricker, V P. Sickle Cell Anaemia, *South Med J* 17: 177, 1924.
- 13 Seydenstricker, V P. Further Observations on Sickle Cell Anaemia, *J A M A* 83: 12, 1924.
- 14 Miyamoto, K, and Korb, J H. Menisocytosis (Latent Sickle Cell Anaemia), *South M J* 20: 912, 1927.
- 15 Lawrence, J S. Elliptical and Sickled Shaped Erythrocytes in the Circulating Blood of White Persons, *J Clin Investigation* 5: 31, 1927.
- 16 Dolgopel, V B, and Stitt, R. Sickle Cell Phenomenon in Tuberculous Patients, *Am Rev Tuberc* 19: 455, 1929.
- 17 Levy, J. Sicklemia, *Ann Int Med* 3: 47, 1929.
- 18 Brandaw, G M. Guidance of Sickle Cell Trait in Industrial Workers, *Am J M Sc* 180 (N S): 813, 1930.

**B. TUBERCULOSIS, Subsurface Growths in Solid Culture Media, Thuringer, J. M., and Butler, H. W.** *Am. Rev. Tuberc.* 31: 466, 1935.

A subsurface growth of the *Mycobacterium tuberculosis* on various solid media has been shown to be a more or less constant phenomenon.

Further evidence is presented that this organism is capable of responding to its environment in many different ways (depending upon conditions of oxygen concentration, reaction of media, temperature, moisture, and its own metabolic products).

The authors are in agreement with those who believe that this organism occurs in various forms and that these pass through a cycle consisting of several phases, but we are unable to demonstrate the factors which bring about these changes.

It is of interest to note that most of the acid-fast rods were found in the subsurface growths, their profusion, arrangement, and acid-fastness depending upon the medium used. This may well lead to the question, "whether this subsurface growth does not resemble the actual status of this organism growing in the tissues more closely than the surface growth."

**HEMORRHAGIC CONDITIONS, Effect of Moccasin Snake Venom in, Peck, S. M., and Rosenthal, N.** *J. A. M. A.* 104: 1066, 1935.

The use of snake venom as a means of controlling various types of hemorrhagic conditions has proved useful. It seems to be of value in most of the types of bleeding not associated with blood changes, such as epistaxis of long standing, uterine bleeding, hereditary multiple telangiectases (Osler's disease) and various forms of purpura, either toxic, allergic or endocrinal. In some instances, marked improvement of the secondary anemia was observed following the control of the bleeding. In some cases of achlorhydric anemia or anemia caused by long-standing uterine bleeding, iron was ineffective until snake venom was employed to control the bleeding.

Snake venom diminishes the bleeding tendency in certain cases of purpura hemorrhagica without affecting the blood picture. In some cases and occasionally in the chronic form, the blood picture returns to normal after the use of snake venom. This is not attributed to the effect of the venom but may be considered to be a spontaneous remission such as occurs without the use of this form of therapy. In such a case, however, the restoration of the blood picture to normal seems to be hastened by the reduction or control of the hemorrhagic condition.

Cases of hemophilia, thrombocythemia and leucemia show either no response or slight response at the beginning of treatment. Apparently snake venom has no appreciable effect on such conditions.

There are no contraindications to the use of snake venom. Allergic responses are rather mild. Exacerbations of the underlying conditions do not occur. Although the fundamental condition may not improve, the treatment does not exaggerate the hemorrhagic state.

Snake venom does not induce any changes in the blood picture. It does not affect the bleeding time, except in certain cases of thrombocytopenic purpura which respond to the treatment. In the allergic state resulting from the injection of snake venom an eosinophilia may supervene. This tends to subside after desensitization.

The administration of moccasin snake venom (*Ancistrodon piscivorus*) intracutaneously or subcutaneously (from 0.2 to 1 c.c. of a 1:3,000 solution) is effective in controlling various hemorrhagic conditions unassociated with blood changes. In thrombocytopenic purpura hemorrhagica, it seems to be effective in some cases and ineffective in others. It is of no value in cases of congenital hemophilia.

**BLOOD, Hemoglobin Index and Jaundice of the New-Born, Hollosi, C., and Horvath, Z.** *Am. J. Dis. Child.* 49: 638, 1935.

The authors' studies lead them to conclude that:

The hemoglobin index of the newborn is less than 100 per cent when jaundice occurs, but more than 100 per cent if jaundice does not occur.

From the hemoglobin index one can foretell whether or not jaundice will occur.

Hemolysis does not necessarily precede jaundice. The formation of bilirubin can be attributed to some as yet unknown histologic function.

calibrated potentiometer until a galvanometer in the plate current indicates that the unknown system is not applying a potential to the control grid. Under such conditions no current is flowing from the electrodes and consequently variations in their resistance have no effect. The disadvantage of such an apparatus is that it requires a rather expensive potentiometer.

Consideration of the above equation shows that the operation of the tube must take place under conditions permitting the minimum flow of grid current. Even when a null point instrument is at perfect balance, a very small current will flow from the grid, since the insulation is never infinitely great. This current disturbs the potential applied to the control grid because it is applied across the high resistance of the glass electrode. Nottingham (1930) gives a very full treatment of the disturbing effect of grid current. The devices of Stadie (1929), Partridge (1929), Elder (1929), Elder and Wright (1928), Schwarzenbach (1930), and many others are subject to error due to the flow of grid current. The grid current was minimized in the devices of Voegtlin, de Eds and Kahler (1930), Muller (1930), Foshbinder (1930), Du Bois (1930), and Partridge (1932).

Metcalf and Thompson (1930) have defined the causes of grid current. An understanding of the factors involved is essential in evaluating the many types of apparatus now available and therefore they will be briefly discussed.

1 *Leakage From the Grid Terminal Over the Surface of the Tube*—This may be minimized by the use of tubes having the control grid contact at the top, and by keeping the glass surface clean and dry.

2 *Ionization of the Residual Gas in the Tube*—Modern tubes are highly evacuated, although traces of gas are always present. Ionization can be prevented by using less than 8 volts on the plate. In actual practice it has been found possible to use 12 volts on the plate, provided the tube is especially "hard." The influence of this factor was reported by van der Bijl (1918).

3 *Emission of Electrons From the Grid Due to Heating*—This is minimized by operating the filament at the lowest practical temperature. The disturbing effect of high temperature filaments has been reported by Kunz (1917), Pike (1919), and by Elder (1929).

4 *Emission of Positive Ions From the Filament*—This is minimized by operating the filament at low temperature. This phenomenon has been studied by Wahlm (1929) and by Smith (1930).

5 *Photo Electronic Emission From the Grid Caused by the Light From the Filament*—This is minimized by operating the filament at low temperature.

6 *Photo Electronic Emission From the Grid Caused by Soft X rays Produced by the Anode Current*—Such effects have been studied by Thomas (1925) and by Richardson (1926).

Perhaps one of the greatest sources of error in the operation of all types of glass electrode apparatus is insufficient insulation of the electromotively active surfaces of the electrode system. This electrical leakage shunts the glass electrode causing a lower potential to be observed. The magnitude of this error depends on the relative magnitudes of the resistance of the glass cell and of the resistance

**SPUTUM TYPING, Reliability of, and Its Relation to Serum Therapy, Bullowa, J. G. M.** J. A. M. A. 105: 1512, 1935.

The pneumococcus obtained from the sputum of patients ill with pneumonia is the type responsible for the disease in over 93 per cent of the cases in which confirmatory evidence was obtained by blood cultures, lung suction, or metastatic foci.

The correct type is obtained from the first sputum in 71 per cent of the cases, from the peritoneum in 49.6 per cent of the cases, from the mouse's heart or brain in 21.4 per cent of the cases, and from the second sputum taken one or two days later in 5 per cent.

The Neufeld reaction gave the pneumococcus type in 76 per cent of cases.

The x group, or "type IV," is found to be responsible for only 1.2 per cent of cases.

The incidence of bacteremia and the death rate are roughly parallel in most types of pneumococcal pneumonia.

Serum reduces the death rate in bacteremic and nonbacteremic cases and prevents bacteremia in some types of pneumococcal pneumonia.

The action is specific and not a general protein therapy.

**ANTICOAGULANTS, Magath, T. B., and Hurn, M.** Am. J. Clin. Path. 5: 548, 1935.

The standard deviation of the hematocrit test as performed with the Sanford-Magath tube is 0.60 per cent.

Heparin produces no swelling, crenation, or laking.

Dry oxalate produces a great deal of shrinkage of erythrocytes. When 22 mg. of dry oxalate per 10 c.c. of human blood is used, the average hematocrit value is 5.16 per cent less than that obtained with wet heparin, which indicates a shrinkage of 11.30 per cent. It is necessary to multiply the value for dry oxalate by 1.127 to equal the true hematocrit.

Sodium oxalate in 1.1 per cent solution gives an hematocrit value equal to that obtained with heparin and does not cause human erythrocytes to swell or become crenated nor does human blood become laked, as observed microscopically and spectroscopically, provided the centrifuging is done before two hours have elapsed. For practical purposes, this anticoagulant may be considered suitable for human blood. If the mixtures of oxalate and blood are allowed to stand the following factors must be used by which to multiply the per cent of erythrocytes: after two hours, 0.980; after four hours, 0.973; and after six hours, 0.969. If 1.6 per cent sodium oxalate be used, one must use the following factors: less than two hours, 1.030; two hours, 1.021; and four hours, 1.019; at six hours, the value is not significantly different from values obtained with heparin.

**NEPHROSCLEROSIS, Malignant, MacMahon, H. E., and Pratt, J. H.** Am. J. Med. Sc. 189: 221, 1935.

Malignant nephrosclerosis from both a clinical and pathologic standpoint should not be looked upon as merely a progression of benign nephrosclerosis, but rather as a distinct and separate disease. It may occur alone or as a terminal complication of the benign disease. In the very early stages, when only the cardiovascular signs and symptoms are present, it may be impossible not only to say whether one is dealing with an early case of benign or malignant nephrosclerosis, but also it may be equally impossible to predict into which of these diseases the case will ultimately fall. As the disease progresses, the renal component becomes more and more conspicuous, and in the late stages it may be impossible to differentiate this disease from chronic glomerulonephritis. The etiology of benign and malignant nephrosclerosis has probably much in common, for one sees cases of chronic lead poisoning, pituitary basophilism, toxemias of pregnancy and so on, which on the one hand may show benign nephrosclerosis and on the other the much less frequent malignant disease. The course and prognosis depend not alone on the quality and quantity of the exciting agent but also in the manner in which the vessel wall responds. Where the response is of a simple degenerative nature, the disease progresses slowly, the prognosis is good and such cases are classed as benign nephrosclerosis. Where the vascular response is characterized by inflammatory changes of the intima, necrosis and hemorrhage, the course is more rapid, the prognosis is poor, and such cases are classed as malignant nephrosclerosis.

The details of Fig 1 are self explanatory. All parts of the apparatus are enclosed in a grounded metal box except the galvanometer and the measuring potentiometer. The parts are mounted directly on the box with their controls external. A small jar of calcium chloride should be sealed inside the box to reduce surface leakages due to moisture. The procedure in operation is as follows:

1 Close switches *S1*, *S2* and *S3* and adjust *R1* so that the voltmeter (*V*) reads 2.5 to 3.5 volts. When tube 89 is used, reduce the filament voltage to the lowest value that will produce a plate current of 0.5 to 0.8 of a milliamperes. Tube 38 should operate at about one half of this plate current.

2 After a few seconds, when the ammeter (*A*) indicates that the plate current has reached its maximum, adjust *R2* and *R3* until the galvanometer reads approximately zero. The grid is now hanging free (at its free grid potential) and consequently the galvanometer is a little unstable, but this does not affect the accuracy of the instrument.

3 Close the tapping switch, *S1*, and adjust *R5* and *R6* until the galvanometer again reads approximately zero. The instrument is then balanced at the free grid potential of the tube. In this condition the galvanometer needle should be steady.

4 Release *S1*, and bridge the calomel cell to the unknown solution containing the glass electrode.

5 Adjust the measuring potentiometer until the galvanometer needle is not deflected when the tapping switch is closed. The needle may be poised at any readable part of the scale. It is only essential that it is not deflected by opening and closing the tapping switch.

The pH of the unknown solution is then calculated from the reading of the measuring potentiometer by the usual procedure.

# REFERENCES

- van der Bijl, H. J. Theory of the Thermionic Amplifier, *Physiol Rev* 12 171, 1918.
- Du Bois, Delafield. A Vacuum Tube Potentiometer Applicable for Use With Glass Electrodes of High Resistance, *J Biol Chem* 88 729, 1930.
- Elder, L. W. pH Measurement With the Glass Electrode and Vacuum Tube Potentiometer, *J Am Chem Soc* 51 3266, 1929.
- Elder, L. W., and Wright, W. H. pH Measurement With the Glass Electrode and Vacuum Tube Potentiometer, *Proc Nat Acad Sci* 14 936, 1928.
- Fosbinder, Russel J., and Schoonover, J. An Improved Method of Measuring Glass Electrode Potentials, *J Biol Chem* 88 605 1930.
- Kunz, J. Amplification of the Photoelectric Current by the Audion, *Phys Rev* 10 205, 1917.
- Metcalf, G. F., and Thompson, B. J. A Low Grid Current Vacuum Tube, *Phys Rev* 36 1489, 1930.
- Morton, Charles. The Effect of Electrical Leakage on the Electromotive Behavior of the Glass Electrode, *J Chem Soc* 1934 236 1934.
- Muller, Friedrich. Theorie und Methodik der Elektronenrohrenpotentiometer zur Messung electromotorischer Kräfte I. *Ztschr f Electrochem* 36 923, 1930.
- Nottingham, W. B. Measurement of Small D. C. Potentials and Currents in High Resistance Circuits by Using Vacuum Tubes, *J Franklin Inst* 209 287, 1930.
- Partridge, H. M. A Vacuum Tube Potentiometer for Rapid E. M. F. Measurements, *J Am Chem Soc* 51 1, 1929.
- Partridge, H. M. A Zero Current Vacuum Tube Galvanometer, *Mikrochemie* 11 337, 1932.
- Pike, Carl F. Amplification of the Photoelectric Current by Means of the Audion, *Phys Rev* 13 102 1919.
- Richardson, O. W. The Excitation of Soft X-rays. *Proc Roy Soc Lond* A110 247, 1926.

Cut thin sections, from 5 to 10 microns, on a freezing microtome and place them in a 1 per cent aqueous solution of sodium cobalti-nitrite for five minutes.

Wash the sections in two changes of distilled water.

Place them in the following (No. 1) solution for fifteen minutes at 67° C.:

Uranium nitrate	1 gm.
Formic acid (chemically pure) 85 per cent	3 c.c.
Glycerin (chemically pure)	5 c.c.
Acetone	10 c.c.
Alcohol, 95 per cent	10 c.c.

Wash in two changes of distilled water.

Place the sections in a 0.75 per cent aqueous solution of silver nitrate for one hour at 67° C.

Rinse rapidly in distilled water.

Place the sections for three minutes in 2 c.c. of the developing solution, to which prior to use add 1 drop of egg albumen-glycerin fixative and thoroughly mix. (The egg albumen-glycerin fixative is that commonly used in fixing paraffin sections to slides.) While developing, work under a 60 watt electric lamp 4 feet above the specimen and expose the developing solution to light about fifteen minutes before it is to be used. The composition of the developing solution is as follows:

Hydroquinone	0.31 gm.
Sodium sulphite	0.006 gm.
Acetone	2.5 c.c.
Solution of formaldehyde (chemically pure) 40 per cent, made neutral	2.5 c.c.
Pyridine	2.5 c.c.
Saturated solution of mastic in 95 per cent alcohol	2.5 c.c.
Distilled water	15.0 c.c.

Wash for a few seconds in distilled water.

Place the sections in the 0.75 per cent solution of silver nitrate previously used from fifteen to twenty-five seconds.

Wash in two changes of distilled water.

Draw the sections onto slides and blot with fine filter paper; dehydrate in absolute alcohol for two minutes and then blot and clear in pure xylene for two minutes.

Mount the section in dammar resin.

#### COMMENT

The sections should be cut quite thin, preferably from 5 to 10 microns in thickness, as thicker sections make demonstration of spirochetes difficult owing to the fat content.

The purpose of using acetone and alcohol in the No. 1 solution is to remove any fatty substance present in the tissue, especially in sections of liver and brain.

The temperature of the oven is important; 67° C. has proved to be optimum. Lower temperatures require a longer time, while 70° C. or above has resulted in failure to demonstrate spirochetes in known control tissue.

Both the No. 1 solution and the solution of silver nitrate should be warmed to 67° C. before sections are placed in them. The No. 1 solution requires fifteen minutes and the solution of silver nitrate about thirty minutes to be heated to 67° C. Therefore, in order to save time it will be good practice to place the solutions in the oven before one begins to cut sections.

Control sections should always be prepared from material known to contain spirochetes.

The developing solution when made up fresh is a creamy color but soon turns brown. The solution keeps well from two to three weeks in a dark cool place. After that it begins to deteriorate, the mastic separating and settling to the bottom of the container. When this occurs, a fresh supply should be prepared.

The No. 1 solution is stable and keeps indefinitely.

Sodium cobalti-nitrite solution is not stable and should be made fresh each time.

All solutions and reagents should be kept in a dark cool place, preferably in a cabinet with black walls.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**HEPATIC FUNCTION, Studies of, in Portal Cirrhosis and Congestive Heart Failure, Cantarow, A Arch Int Med 56 521, 1935**

Hyperbilirubinemia or abnormal retention of bromsulphalein or both were present in seventeen of twenty two patients with portal cirrhosis. Visible jaundice was present in eight of twelve patients with hyperbilirubinemia.

Although ascites usually precedes the development of visible icterus, varying degrees of hyperbilirubinemia may be present in many cases before ascites can be demonstrated.

Retention of bromsulphalein was noted in 77.3 per cent of the cases in this series, being at times exhibited by patients with normal serum bilirubin values (seven cases). The relationship between retention of dye and the presence of ascites was not as consistent as was the relation of the latter to the degree of hyperbilirubinemia.

This dissociation of the retention of dye and that of bilirubin is practically never encountered in obstructive types of hyperbilirubinemia and suggests that primary impairment of the function of the hepatic cells is of fundamental importance in the production of hyperbilirubinemia in cases of portal cirrhosis. Similar observations were made in four cases of splenic anemia (Banti's disease).

Hyperbilirubinemia was present in ten of forty two cases of congestive heart failure, in only two of which there was visible jaundice. Abnormal retention of bromsulphalein was present in fourteen cases. The severity of the condition appeared to be more consistently related to the degree of retention of dye than to the concentration of serum bilirubin.

Marked impairment of excretion of dye may occur in the absence of hyperbilirubinemia. Although not conclusive, this observation suggests that some factor, probably pulmonary in nature, other than hepatic functional impairment is necessary for the production of hyperbilirubinemia in patients with congestive heart failure.

**BLOOD Standards for Normal Children of School Age, Osgood, E. E. and Baker, R. L. Am J Dis Child 50 343, 1935**

Hematologic examinations are reported on the oxalated venous blood of 112 healthy boys and 103 healthy girls, aged from 4 to 13 years, inclusive.

The accurate methods used in making these examinations are briefly discussed.

There were no significant differences in these hematologic examinations with age or sex in this group.

The following values are recommended as normal for children of this age:

	Average	95 Per Cent Range
Erythrocyte count, millions	5	4.20 to 5.80
Hemoglobin, grams	12	10.00 to 14.00
Hemoglobin, coefficient	12	10.20 to 13.80
Cell volume, c.c.	36	31.00 to 41.00
Volume coefficient	36	31.00 to 41.00
Color index	1	0.85 to 1.15
Volume index	1	0.85 to 1.15
Saturation index	1	0.90 to 1.10

The results in these 215 children agree satisfactorily with those summarized from the literature.

The application of these normal standards to the diagnosis and treatment of anemia in children are discussed.

of patients seek the physician's advice because of signs and symptoms which in themselves may be of minor degree, though not always of minor significance.

In this book Dr. Pardo-Castello has excellently described disturbances of the nails, sometimes of importance as diseases of the nails per se, sometimes significant of underlying and more generalized disturbances, but always of importance to the patient.

Many of these without doubt are at times overlooked by the physician; many others, though seen, fail to receive due recognition of their importance or significance, largely because accurate information concerning them is likely to be the exception rather than the rule.

This may well be due to the fact that the nails and their disturbances have received heretofore relatively little mention in medical literature, a hiatus which this book completely fills.

Commencing, logically, with a discussion of the anatomy, physiology, and pathology of the nails, the text then discusses the affections peculiar to the nails, onychodystrophies, the ungual manifestations of dermatoses and of systemic diseases, congenital affections of the nails, and the roentgen ray treatment of diseases of the nails.

An addendum lists the occupations in which diseases of the nails commonly occur and the ungual symptoms due to poisons.

All in all this is a well-written, comprehensive, and excellently illustrated text which the physician may read with profit and which he will find most useful as a reference.

### A Textbook of Pathology\*

ONE of the finest textbooks of pathology comes out entirely reset in its sixth edition, with new material gleaned from the last four years, especially plentiful in the fields of vitamins, viruses, and endocrine functions. Dr. MacCallum's treatment of diseases as entities with their accompanying pathological alterations gives a fine concept of the diseases as pathological wholes in contrast with the treatment of pathological alterations as entities with accompanying lists of conditions in which they are found, preferred by some authors. This book will continue, by means of the new edition, to be an invaluable textbook for the student of both pathology and bacteriology.

### Tissue Immunity†

DR. KAHN presents in this book original experimental studies dealing with the reactivity of the tissues themselves to the injection of foreign substances. Although it has long been known that an animal can be immunized by the injection of increasing amounts of organisms or their products and this immunity measured by humoral methods, Dr. Kahn has gone further and endeavors to tell us what tissue changes underlie the activities constituting this immunity. Likewise, desensitization of allergic persons has been practiced for some time, but it is the actual changes in the activity of the tissues producing the states of hypersensitivity and desensitization that have been studied here.

In each chapter there are experiments described with their results tabulated, and the theoretical considerations arrived at by the author from these results, which should be of great interest to the teacher, the research man, and the student of immunology. Following these are clinical considerations to aid the inquiring clinician in his understanding of the actual activities he is producing in the ever-increasing modes of prophylaxis, therapy, and diagnosis by parenteral methods.

\*A Textbook of Pathology. By W. G. MacCallum, Professor of Pathology and Bacteriology, The Johns Hopkins University, Baltimore. Sixth Edition. Entirely reset. 1277 pages with 697 illustrations, cloth. W. B. Saunders Company, Philadelphia and London, 1936.

†Tissue Immunity. By Reuben L. Kahn, M.S., D.Sc., University of Michigan, Ann Arbor. Cloth, 707 pages. Charles C. Thomas, Springfield, Ill., 1936.

**LYMPHOGRANULOMA INGUINALE, Value of Intradermal Injection of Serum as a Diagnostic Test for,** Haynes, H A Arch Dermat & Syph 32 795, 1935

Five patients with lymphogranuloma inguinale showing positive reactions to Frei antigen were tested under adequate conditions of control with intradermal injections of serum from patients known to have lymphogranuloma inguinale

The mixture of Frei antigen and serum from a patient with lymphogranuloma inguinale produced the same cutaneous reaction as that to Frei antigen alone

The intradermal injection of serum from patients with lymphogranuloma inguinale into patients showing positive reactions to Frei antigen caused no reaction, with one doubtful exception

Therefore, it seems that the substitution of serum for Frei antigen is not satisfactory as a diagnostic procedure in cases of lymphogranuloma inguinale

**HEMATOPOIETIC EQUILIBRIUM, The, and Emergency Splenectomy,** Doan, C A, Curtis, G M, and Wiseman, B K J A M A 105 1567, 1935

The pathologic physiology of the spleen may be manifest through either or both of two mechanisms (a) inhibitory, (b) destructive—and may affect any or all of the circulating blood elements

The spleen is the major pathologic agent in congenital hemolytic jaundice

Splenectomy is indicated as a prophylactic measure against clinical exacerbations of excessive hemolytic activity in the chronic and subacute manifestations of the disease

Splenectomy is also the therapeutic procedure of choice in acute hemoclastic crises, whether the crisis is of spontaneous or of precipitated origin, and regardless of the severity of the anemia

The immediacy of the erythrocyte response following splenectomy in hemolytic jaundice is dramatic, occurring on the operating table. It is usually a million or more cells per cubic millimeter in quantity and represents a true increase in total available circulating units. Thus autotransfusion removes the necessity for preoperative and/or postoperative transfusions

Splenectomy is not contraindicated in properly selected cases of thrombopenic purpura in acute crisis, provided adequate preoperative blood transfusions are given. The immediacy of the beginning recovery and reappearance of blood platelets in the circulation following splenectomy in thrombopenic purpura may be quite as dramatic as the changes noted in hemolytic jaundice

**PLASMA CHOLESTEROL, Concentration in Glomerulonephritis and Other Terminal States,** Cantarow, A, and McCool, S G Am J Clin Path 5 516, 1935

Determinations were made of the plasma cholesterol concentration in 18 patients (14 of whom died) with advanced chronic glomerulonephritis, 32 with nonnephritic nitrogen retention (26 of whom died) and 18 dying of conditions not accompanied by nitrogen retention. Although there was a distinct tendency toward fixation of the cholesterol concentration at a low level with increasing grades of nitrogen retention in both the nephritic and the nonnephritic groups, there was no constant quantitative relationship between the degree of cholesterolemia and of nitrogen retention. Low values were usually obtained in the group of terminal states not associated with nitrogen retention. No constant relationship was noted between the degree of hypocholesterolemia and of anemia

The fact that similar findings are obtained in a variety of terminal states suggests that the development of hypocholesterolemia and its serious prognostic significance, under such circumstances, are probably related to the operation of some fundamental mechanism which is stimulated by a variety of pathologic states. Certain observations are reported which suggest that excessive withdrawal of cholesterol from the blood, as a result of abnormal stimulation of the activity of the reticulo-endothelial system, may be of importance in the pathogenesis of this phenomenon

# Venereal Disease Information

A monthly publication prepared by the U. S. Public Health Service for distribution among the medical profession throughout the United States. It measures approximately 6 by 9 inches and ranges in size from 25 to 75 pages.

It is the purpose of the Public Health Service in issuing this publication to provide in condensed form a monthly summary of the scientific developments in the diagnosis, treatment, and control of syphilis and gonorrhea. More than three hundred American and foreign journals are reviewed for this work. Abstracts are made of articles describing laboratory, pathologic, and clinical work in the field of venereal diseases.

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The cost of this publication is only fifty cents per annum, payable in advance to the Superintendent of Documents, Government Printing Office, Washington, D. C. It is desired to remind the reader that this nominal charge represents only a very small portion of the total expense of preparation, the journal being a contribution of the Public Health Service in its program with State and local health departments directed against the venereal diseases. If you wish to secure the valuable service which this monthly magazine provides, send fifty cents to the Superintendent of Documents, Government Printing Office, Washington, D. C.

## Lehrbuch des Stoffwechsels und der Stoffwechselkrankheiten.

Von Dr. med. et phil. S. J. Thannhauser, o. ö. Professor der Medizin, Direktor der Medizinischen Klinik der Medizinischen Akademie Düsseldorf. Mit 94 teils farbigen Abbildungen im Text. XX, 741 Seiten. 1929. RM 51.12; gebunden RM 53.82

This volume is a notable work in that it succeeds, to an extent rarely attained, in presenting a very detailed and authoritative account of the known facts of metabolism, without becoming a mere collection of abstracts from the literature. The book is in the German tradition in that it contains much controversial matter, that no pains have been spared to obtain the most recent advances in the various sections, and that its outlook is both scientific and philosophical. Professor Thannhauser has the great advantage of combining a very complete knowledge of the natural sciences with a fine and experienced clinical sense. This combination is not common, and we greet the present volume as a demonstration of the immense advantages gained by the clinician who possesses a thorough scientific training. The treatment of the various subjects is well thought out, and shows the hall marks of a teacher. . . . . Many and excellent photographs of cases and diagrams of results abound, and the bibliography makes the volume of incalculable value to the researcher. A translation into English would be of great service to a wider public.

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**PREGNANCY, Investigation of New Biologic Test for Hormones in Pregnancy Urine**  
 Kliener, I S, Weisman, A I, and Barowsky, H J A M A 104 1308, 1935

Group 1 Twenty one female bitterlings were tested with known pregnancy urines. Of these, eight tests were negative, nine were definitely positive, and four showed some enlargement of the ovipositor.

Group 2 Urines from seven normally menstruating nonpregnant women were tested by the fish method, with the result that four gave positive ovipositor tests.

Group 3 Samples of urine from four different normal males were tested, with one positive result. This positive result was so striking and occurred so rapidly (before forty eight hours) that the authors obtained another specimen from the same individual, a healthy normal man, aged 21. This was tested on six fish in separate aquariums with positive results in every fish within forty eight hours.

Group 4 Three samples were obtained from women who were in the postclimacteric period and had not menstruated for from four to seven years. Each urine of this group was tested on two fish. One urine gave a positive fish test.

Group 5 The urines of Group 1 (from known pregnancies) were boiled and tested in the same way. In some cases two boiled urines were mixed and tested. The results were quite irregular, some boiled urines giving positive reactions and some negative.

It will be seen that only nine of the twenty one urines from pregnant women gave definitely positive reactions. Of seven from normally menstruating nonpregnant women, four gave positive reactions. One male urine of the four tested was positive, and a later specimen from the same male gave positive reactions in every one of the six fish tested. Urines from women who had passed the menopause were positive in one of three cases. Boiled urines from pregnant women were positive in some instances and negative in others.

These results agree with those of Szusz and indicate that this biologic reaction is not a specific test for pregnancy as Kanter, Bauer, and Klawns intimate. Moreover, the latter authors do not report as great a variety of controls as we have and as Szusz previously reported. The author's finding that nonpregnant female urines, postmenopausal urines, male urines, boiled pregnancy urines, and physical disturbance may bring about lengthening of the ovipositor would seem to show that a standardization of a fish, as these authors suggest, would be extremely difficult if not impossible. Whether the phenomenon is due to the presence of estrogenic substance or to some other hormone or to some other substance remains to be determined.

**TISSUE, Application of Blood Staining to Formalin Fixed Tissues, Wilder, H C J Techn Meth & Bull Int Assn Med Mus 14 68, 1935**

- 1 Xylol
- 2 Absolute methyl alcohol, 2 changes
- 3 Cover slide with a freshly filtered saturated solution of Jenner's stain (National Aniline and Chemical Co.) in methyl alcohol, 3 minutes
- 4 Add an equal amount of distilled water—1 minute
- 5 Plunge, without washing, into a Coplin jar of dilute Giemsa solution (1 drop of Giemsa's spirochete stain from Hynson, Wescott and Dunning to 1 c.c. of distilled water)—45 minutes
- 6 Rinse and differentiate in acidulated distilled water (1 drop of glacial acetic acid to 5 c.c. of distilled water)
- 7 Rinse in pure distilled water
- 8 Dehydrate quickly in 95 per cent alcohol and two changes of absolute alcohol
- 9 Clear in xylol and mount in Canada balsam

**TISSUE, Rapid Staining Method for Spirocheta Pallida in Single Sections, Krajian, A A Arch Dermat & Syph 32 764, 1935**

Drop fresh blocks of tissue about 5 mm thick in a 10 per cent solution of formaldehyde heated to 67° C (152.6° F) and fix in an oven at 67° C for ten minutes. (Tissues already fixed with formaldehyde do not require this treatment.)



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## REVIEWS

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### The Clinical Use of Digitalis\*

DIGITALIS was an ancient remedy long before the publication of William Withering's epochal book, *An Account of The Foxglove and Some of Its Medicinal Uses*. That it is a valuable one is evidenced by the extensive literature which has accumulated concerning its characteristics and effects and the indications and contraindications to its therapeutic use.

The purpose of this book is to present clearly the various problems attendant upon the clinical use of digitalis and to survey the various data applicable to their solution.

The scope of the book is indicated by the Table of Contents: Effect on Ventricular Muscle, Effect on A V Tissues, Effect on the Pacemaker of Normal Rhythm, Diuretic Effect, Effect on Blood Vessels and Blood Pressure, Electrocardiographic Effects, Toxic Effects, Indications, Use in Certain Special Situations, Dosage and Method of Administration, Contraindications and Dangers, and Digitalis and Prognosis.

The final chapter, "Therapeutic Theses," sums up concisely the conclusions regarding the clinical use of digitalis which at present appear to be indicated by the evidence presented in the preceding chapters. "If this has sometimes involved a measure of dogmatism unwarranted in reasoned discussion, the author trusts that these theses may yet commend themselves to thoughtful consideration and appropriate consideration."

These may be thus summarized: The therapeutic action of digitalis results from the action of the drug upon ventricular muscle, which muscular effect is of therapeutic value in "potential" heart failure. Digitalis lessens the energy requirement of the failing myocardium, it does not force it to work harder, but enables it to work better.

The indication for digitalis lies not in the manifestations of heart failure but in the heart failure itself.

Digitalis therapy must be considered primarily with reference to heart function, not to heart rhythm.

Diuresis from digitalis is a general circulatory effect, blood pressure changes are secondary circulatory effects. Pneumonia, shock, toxic states and septicemia are not indications for digitalis. Nor is angina pectoris.

While there are numerous other theses, based upon the data presented in this book, enough have been summarized to show that Dr Luten has written an eminently practical book which should prove of great interest and still greater value to the physician.

Both author and publisher may be congratulated upon this book and, perhaps, most of all the physician who may find it in practical help in the solution of some harassing clinical problem.

---

### Diseases of the Nails†

IT IS, perhaps, a truism to say that the recognition of disease connotes the detection, recognition, and correlation of minutia, just as it may also be true to recall that the majority

---

\*The Clinical Use of Digitalis. By Drew Luten M.D. Associate Professor of Clinical Medicine in the Washington University School of Medicine and Physician to Barnes Hospital St. Louis. Cloth 226 pages two plates Charles C Thomas Springfield Ill.

†Diseases of the Nails. By V Pardo Castello M.D. formerly Assistant Professor of Dermatology and Syphilology University of Havana with a foreword by Howard Fox M.D. Professor of Dermatology and Syphilology New York University. Cloth 177 pages 24 illustrations Charles C Thomas Springfield Ill.

# ENDOCRINOLOGY

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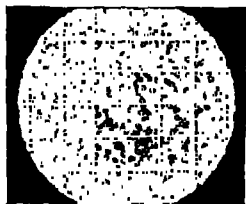
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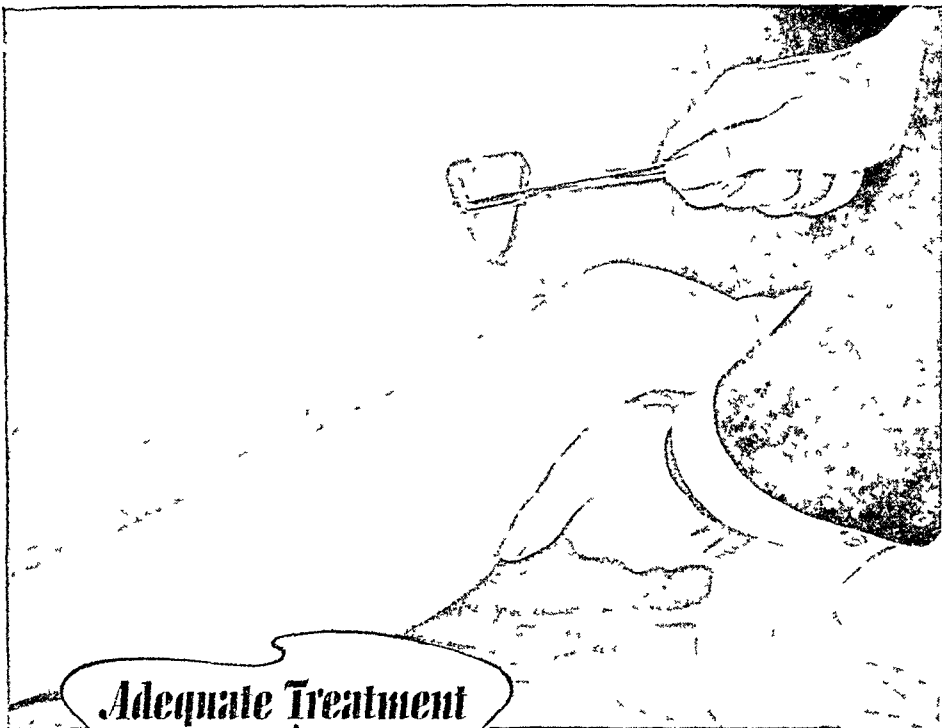
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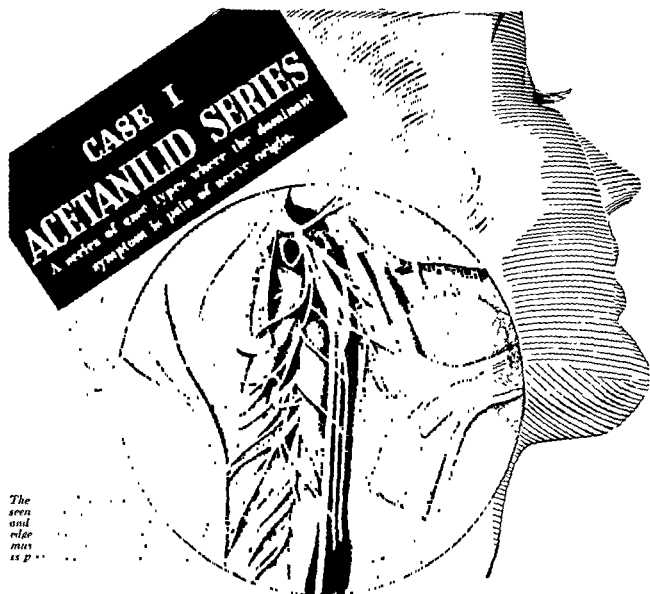
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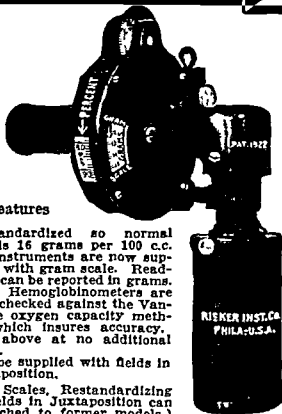
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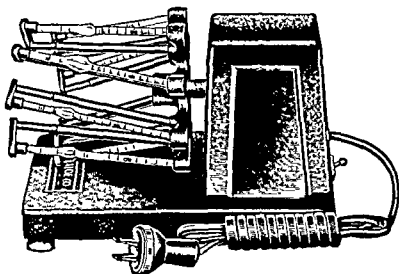
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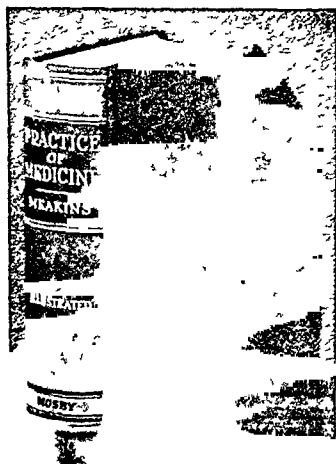
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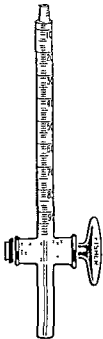
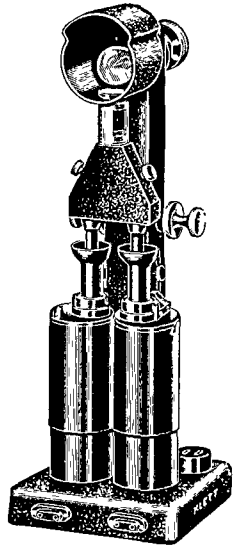
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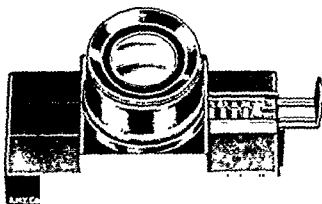
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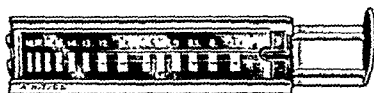
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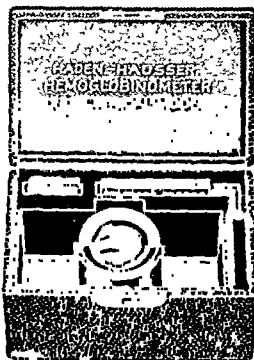


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<sup>1</sup>See Russell L. Haden, "A New Hemoglobinometer," *The Journal of Laboratory and Clinical Medicine*, Vol. XVI, No. 1 (October, 1930), p. 68.

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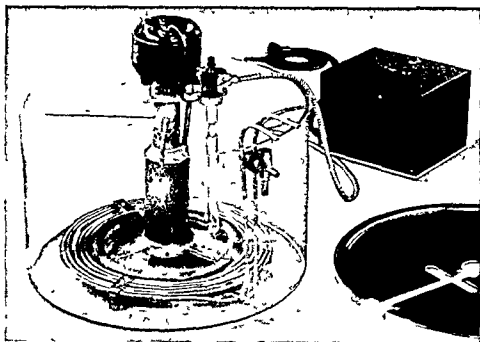
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ber normally in the circulation shows little variation. The life history of the erythrocyte is shown in Table I. To form red cells, nonspecific substances

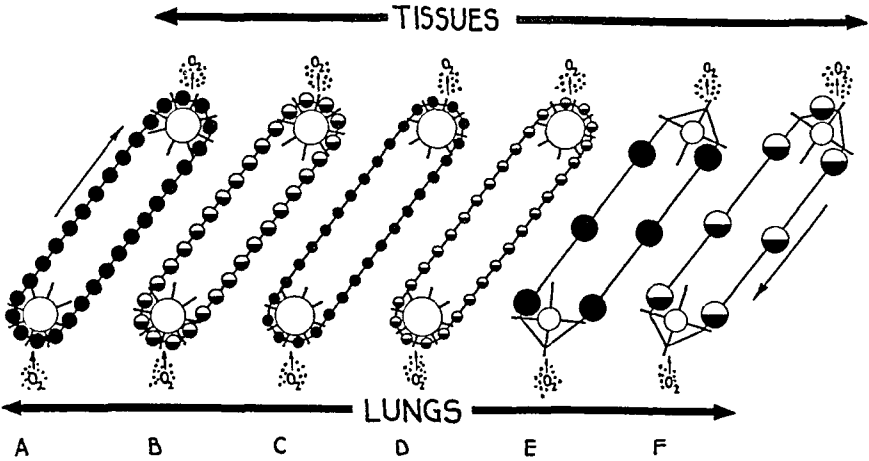


Fig. 1.—Schematic illustration of red cells functioning as units (cups) on an endless-chain conveyor. A, Normal red cells. B, Red cells of normal size partly filled with hemoglobin (normocytic, hypochromic anemia). C, Small red cells completely filled with hemoglobin (microcytic, hypochromic anemia). D, Small cells, partly filled with hemoglobin (microcytic, hypochromic anemia). E, Large cells, completely filled with hemoglobin (macrocytic, hyperchromic anemia). F, Large cells containing a normal amount of hemoglobin (macrocytic, normochromic anemia).

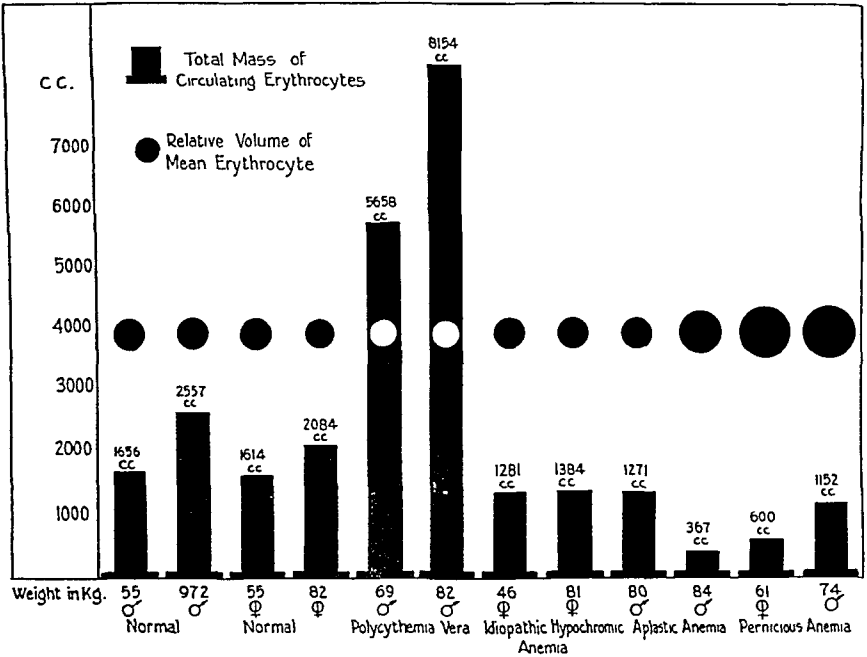


Fig. 2.—Variation in total mass of circulating red cells in various conditions. The circle indicates the relative volume of the unit of mass (the red cell) in each instance.

necessary for building all cells are needed. These are protein, fat, carbohydrate, water, vitamins, and mineral salts. Two specific substances are also



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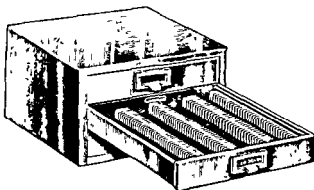
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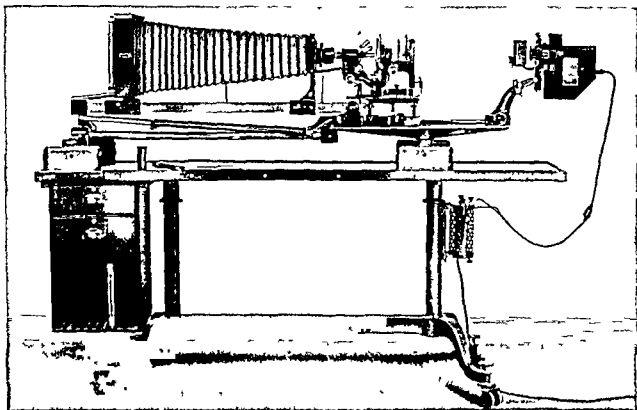
the amount of bilirubin present in the plasma is an indicator of the rate of red cell and hemoglobin destruction. A correlation of the bilirubin content of the plasma and the reticulocyte level is shown in Table II.

TABLE II

RELATION OF BLOOD FORMATION AND DESTRUCTION TO BILIRUBIN AND RETICULOCYTE LEVEL

BILIRUBIN CONTENT (ICTERUS INDEX)	RETICULOCYTE COUNT		
	INCREASED (OVER 1.5%)	NORMAL (0.5-1.5%)	DECREASED (UNDER 0.5%)
Increased (over 6 units)	Increased blood destruction with active bone marrow	Increased destruction without good marrow response	Increased destruction with inactive bone marrow or impaired delivery of red cells
Normal (4-6 units)	Active bone marrow without excessive destruction	Decreased marrow without excessive destruction of red cells	Decreased formation or impaired delivery of red cells without excessive destruction
Decreased (under 4 units)	Decreased destruction of hemoglobin due to iron deficiency; active cell formation in marrow	Decreased destruction of hemoglobin. Decreased formation of hemoglobin; normal cell formation in marrow	Decreased destruction of hemoglobin. Decreased formation of hemoglobin. Decreased cell formation in marrow or impaired delivery of cells

If all elements necessary for red cell formation are deficient, the marrow cannot make the normal number of cells at the normal rate. The marrow functions at a low rate of speed but such cells as are delivered into the circulation are usually normal. The two specific elements, iron and erythrocyte maturing factor (EMF), are necessary if the marrow is to make a normal cell with a normal complement of hemoglobin. As the red cells develop in the bone marrow, they multiply actively at the megaloblast stage but are not ready for delivery from the marrow until completed by a substance formed by the interaction of a secretion of the stomach (the intrinsic factor of Castle) on food elements (the extrinsic factor of Castle) and stored in the liver. This substance has been designated by many names, as "liver principle," the "antianemic principle of Castle," the "pernicious anemia principle," and "antimegalocyte principle." Its fundamental action is to mature the red cell, or prepare it for emergence from the marrow, so we have designated it erythrocyte maturing factor (EMF). Since it is always necessary to know, in studying an anemia, whether there is a sufficient supply of this essential factor, we must have some indicator of its lack. The cell to which this substance (EMF) is supplied becomes smaller so a decrease in volume of the cell is characteristic of the maturation effected by the erythrocyte maturing factor and a macrocytosis is indicative of its lack. While a macrocytosis is usually an indicator of a deficiency of the erythrocyte maturing factor (EMF), cells of increased size may be due to other causes. A hyperplastic marrow, overactive in response to a great demand for red cells, may deliver red cells larger than normal. These are large because of rapid removal from the marrow before maturation is complete rather than a lack of erythrocyte maturing substance (EMF). Thus the hyperplastic marrow in response to rapid destruction of red cells in phenylhydrazine poisoning or in spherocytic



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or delivery of red cells (the reticulocyte count), the lack of the erythrocyte maturing factor or EMF (macrocytosis), and a deficiency of iron (hypochromia and microcytosis) as tabulated in Table III. I have described elsewhere the technic of the blood examination to supply such data.<sup>2</sup> A careful laboratory study is first necessary in every anemia to furnish the data outlined above. From the laboratory examination the anemia is classified on the basis of the number, size, and hemoglobin content of the mean red cell.<sup>3</sup> These studies are illustrated in Fig. 3. The relation of the blood findings to red cell formation and destruction is shown in Table IV.

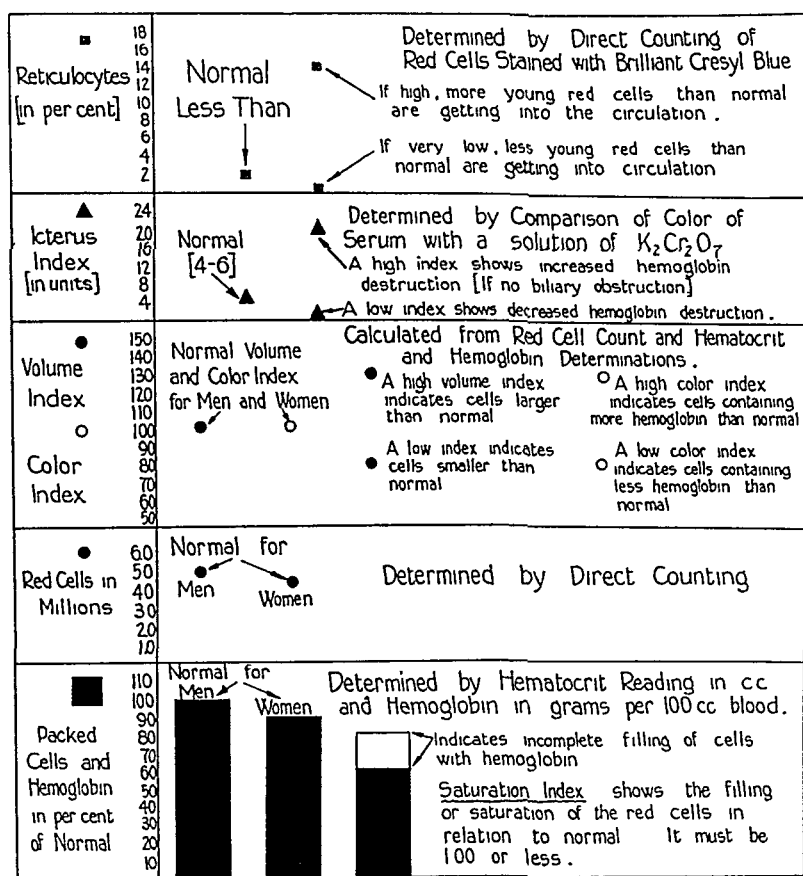


Fig 3.—Study of blood and interpretation of findings in anemia

Since an anemia represents a loss of balance between red blood cell formation and destruction, an anemia can result only from increased blood loss without a compensating increase in blood formation, by decreased formation with a normal or accelerated blood loss, or by a combination of increased blood loss and decreased formation. A clinical classification of anemia on the basis of method of production with the more important clinical causes is given in Table V. In every anemia it is necessary to make both a laboratory and clinical classification.

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## *CLINICAL AND EXPERIMENTAL*

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### THE MECHANISM OF ANEMIA\*

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ANEMIA is a reduction below normal of the capacity of the blood to transport the oxygen necessary for all animal life. The body tissues must be supplied with many times as much oxygen as can be carried in physical solution in the plasma. The hemoglobin normally present (15 to 16 gm per 100 cc of blood) increases one hundred times the power of the blood to transport oxygen by carrying it in chemical combination. This amount of hemoglobin in solution in the circulating blood would greatly increase the osmotic pressure of the plasma beyond that of the surrounding tissues and so dehydrate the tissues. Hemoglobin in a red cell is outside the plasma, does not affect the osmotic pressure and yet functions efficiently as an oxygen carrier since absorption and release of oxygen is as efficient as if the hemoglobin were in solution in the plasma. The red cell is thus simply a container<sup>1</sup> for the necessary hemoglobin and functions as a cup on an endless chain conveyor. It is normally filled with hemoglobin and is constantly making round trips from the lungs to the tissues. This conception of the function of the red cell applied to the different laboratory types of anemia is illustrated in Fig. 1. In addition to thinking of the red cell as a cup on an endless chain conveyor we should also visualize the total mass of circulating red cells as a vessel containing hemoglobin. The size of this vessel varies enormously in blood disorders affecting the red cell as illustrated in Fig. 2.

The problem of anemia is primarily concerned with hemoglobin and its carrier the red cell. The span of life of a red cell averages thirty days. About a trillion red cells are formed and destroyed each day since the num

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\*From the Cleveland Clinic

The cells remaining in the circulation are normal and the process of formation and destruction is unaltered. This state persists for only a short time, however, after the hemorrhage, when there is increased activity of the marrow to compensate for the blood lost. The icterus index falls (2 units) and the amount of bilirubin and iron set free decreases, and the picture is now one

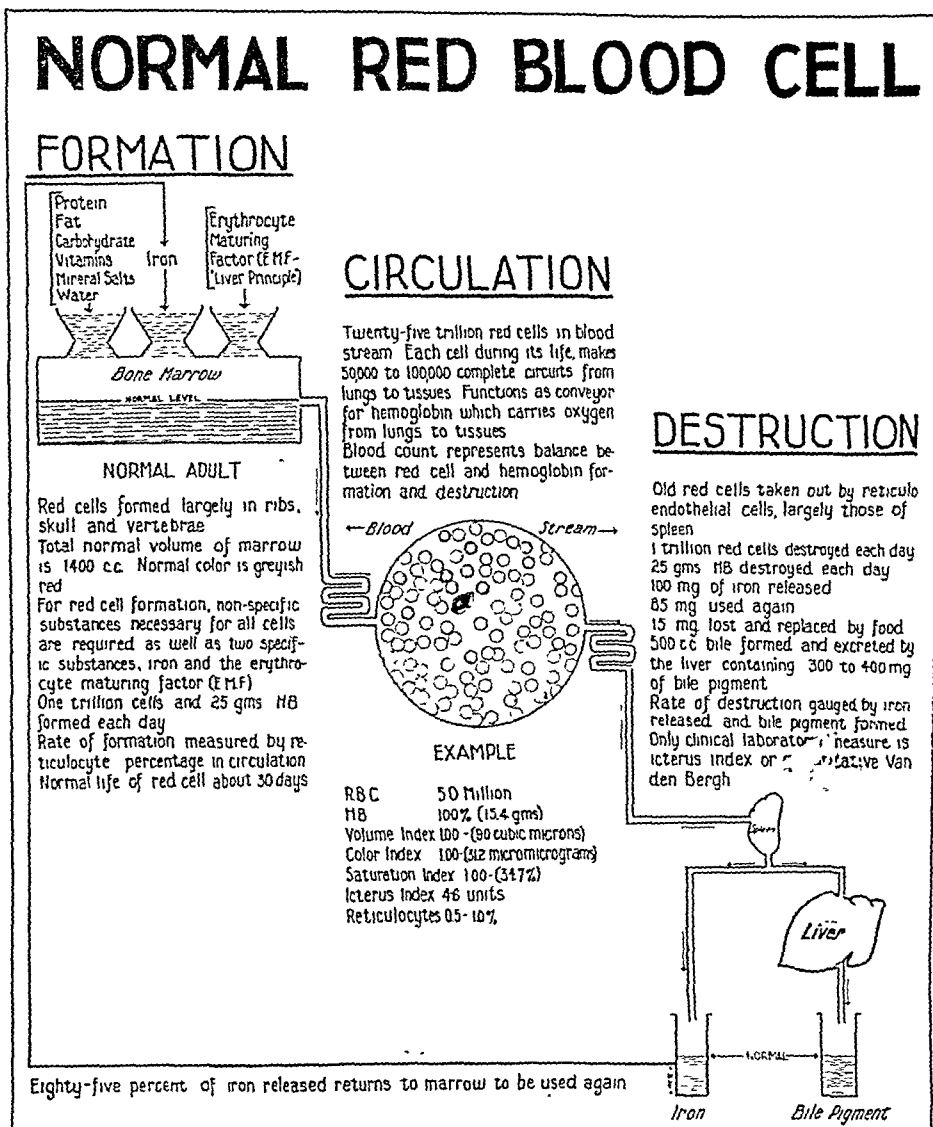
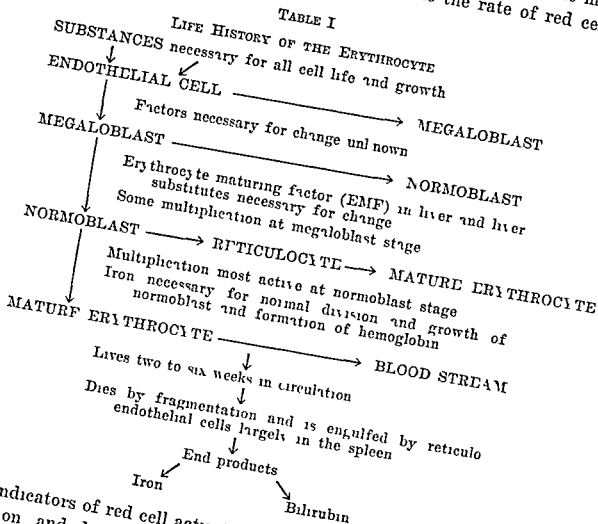


Fig. 4.—Normal red cell physiology.

of an iron deficiency anemia, shown in Fig. 6, where there is a defect in the supply of iron to the marrow with the result that the cells are small (volume index 0.067) and have a decreased hemoglobin content (color index 0.4). The therapeutic indication is to stop the blood loss and supply an adequate amount of iron.

required, the iron necessary for hemoglobin formation and the substance supplied by liver and liver substitutes necessary for the maturation of red cells. To evaluate an anemia, we must understand red cell formation in the marrow, know the level of circulating elements, and visualize the rate of red cell and hemoglobin destruction.



Certain indicators of red cell activity are necessary to evaluate the formation circulation and destruction of red cells. The red cell count and the hemoglobin content record only the balance between red cell formation and red cell destruction. Young red cells have the property of staining with certain dyes before they are fully matured. The number of reticulocytes or young cells which take this stain is an index of the rate of production of cells ready to function in the blood stream or at least the rate of delivery of such cells from the marrow. The marrow may be hyperplastic or hyperactive with a low reticulocyte count in the circulation if the delivery of cells from the marrow is impaired. If the reticulocyte count in the circulation is high, the marrow is necessarily hyperplastic, if below normal the marrow may be aplastic, hypoplastic, or hyperplastic.

When a red cell is destroyed, hemoglobin is set free. Iron is split off from the hemoglobin molecule, and bilirubin is formed as the end product of the pigment metabolism. Bilirubin so formed is adsorbed by protein and is not easily excreted by the kidney. The capacity of the liver cells to excrete bilirubin so formed is also quickly exceeded, so an excessive destruction of red cells and hemoglobin is soon reflected in an increased bile pigment content of the plasma. In the absence of biliary obstruction and liver disease,

In Fig. 7 is illustrated the red cell mechanism in an anemia due to excessive hemolysis resulting from the improper use of phenylhydrazine. This patient was given this drug on the basis of a wrong diagnosis of polycythemia vera. The polycythemia was a symptomatic one since the blood volume was normal. When first seen, the patient had a well-marked anemia (hemoglobin 58 per cent) with a high icterus index (25 units) and a high reticulocyte count (10.3 per cent). The bone marrow here is overactive and increased in volume as indicated by the reticulocytosis to compensate for the excessive red cell destruction. The supply of building materials is normal. The cells damaged by the phenylhydrazine are rapidly removed from the circulation so the spleen is overactive and larger than normal. The output of iron and bile

## RED CELLS IN ANEMIA DUE TO HEMOLYTIC AGENT AS PHENYLHYDRAZINE

### FORMATION

### CIRCULATION

### DESTRUCTION

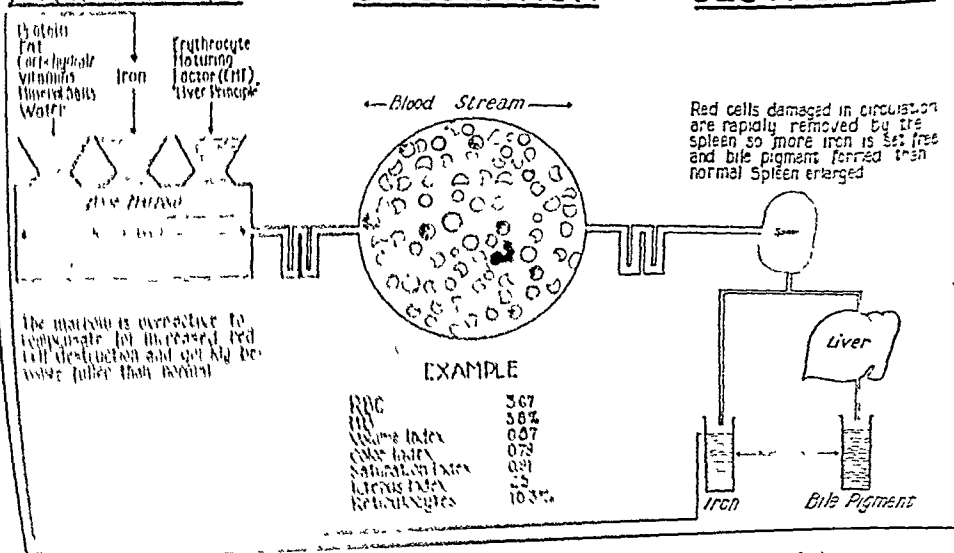


Fig. 5. Pathology of red cells with excessive destruction by a toxic substance.

is necessarily greater than normal. In this patient the primary difficulty is the damage to the red cells in the circulation, so the therapeutic indication is to stop the cell damage. There is no need for iron, liver preparation, or marrow stimulation unless the process has proceeded to the point of exhaustion. In this instance, after the action of the drug was past, the spleen, by its action, the tendency to remove the red cells, and hemoglobin all returned to normal.

In the case of the red cell mechanism in congenital hemolytic icterus and splenic enlargement, this patient had a well-marked anemia with a high icterus index, a high reticulocyte count, excessive blood destruction and a high reticulocyte count, all of which were due to the same cause. The supply

jaundice may deliver macrocytic cells. A chronic hyperplasia of marrow in response to increased cell loss usually leads in time, however, to the formation of cells smaller than normal. Iron is the second specific element necessary for normal red cell formation. Without iron hemoglobin cannot be formed. It is most probable also that iron stimulates the growth and multiplication of red cells at the normoblast stage where division is most active. With a decrease in the normal amount of hemoglobin in the blood, there is first a decrease in the concentration of hemoglobin in the red cells or decreased color index. Since there is no value in having red cell stroma without hemoglobin to fill it if the color index continues low the cells become smaller and the volume index decreases. The hypochromia shown by the lessened color index and volume index is a measure of the lack of iron.

Thus we have accurate indicators to show the balance between red cell formation and cell destruction (the red cell count and hemoglobin content), the rate of destruction of red cells (the icterus index), the rate of regeneration

TABLE III  
MEASURES OF RED CELL ACTIVITY

FACTOR	INDICATOR
Balance of red cell and hemoglobin formation and destruction	Red cell count and hemoglobin content
Rate of destruction of red cells	Level of bile pigment in plasma
Rate of regeneration of red cells	Level of reticulocytes in circulation
Deficiency of iron	Hypochromia and microcytosis of red cells
Deficiency of erythrocyte maturing factor (EMF)	Macrocytosis of red cells

TABLE IV  
RELATION OF BLOOD FINDINGS TO RED CELL FORMATION AND DESTRUCTION

Active bone marrow	{ Increased number of reticulocytes basophilia nucleation Slight increase in mean erythrocyte volume if reticulocytosis is marked. Often an increase in leucocytes and platelets unless destruction is more active than normal. The number of cells is increased.
Inactive bone marrow	{ Decrease or absence of reticulocytes basophilia and nucleation. If blood destruction is normal or increased, the cell count decreases.
Increased red cell and hemoglobin destruction	{ Increase in bilirubin content of plasma decrease in number of cells unless compensated for by increased marrow activity.
Decreased hemoglobin destruction	{ Decrease in bilirubin content of plasma.
Deficiency in erythrocyte maturing factor (pernicious anemia)	{ Anemia with increase in mean erythrocyte volume (increased volume index).
Deficiency in iron (iron deficiency anemia, chronic hemorrhagic anemia)	{ Anemia with hypochromia of red cells (decreased color index), microcytosis (decreased volume index) if hypochromia continues.
Hemolytic anemia	{ Anemia with increased icterus index, reticulocytosis if marrow responds to increased need.
Anemia due to decrease in amount or activity of marrow (aplastic or hypoplastic anemia)	{ Anemia with cells of normal size and hemoglobin content, decrease in reticulocytes.

excessive numbers of abnormal cells from the circulation. Splenectomy helps the anemia<sup>5, 6</sup> but here the result differs from that seen in spherocytic anemia in that the patient continues to have some anemia after removal of the spleen. The increased cell destruction and formation also continue so the excessive activity of the spleen cannot be the sole cause of the anemia. It is most probable that the red cells fragment more easily than normal and this fragmentation continues after splenectomy. There is no treatment for this phase of the disorder. Splenectomy removes only one factor in the anemia.

Fig. 10 illustrates the anemia due to marrow aplasia caused by the prolonged use of arsphenamine. The amount of functioning marrow tissue is decreased. In this instance the blood examination shows a marked anemia

## RED CELLS IN ANEMIA DUE TO SICKLE SHAPE OF CELLS

### FORMATION

### CIRCULATION

### DESTRUCTION

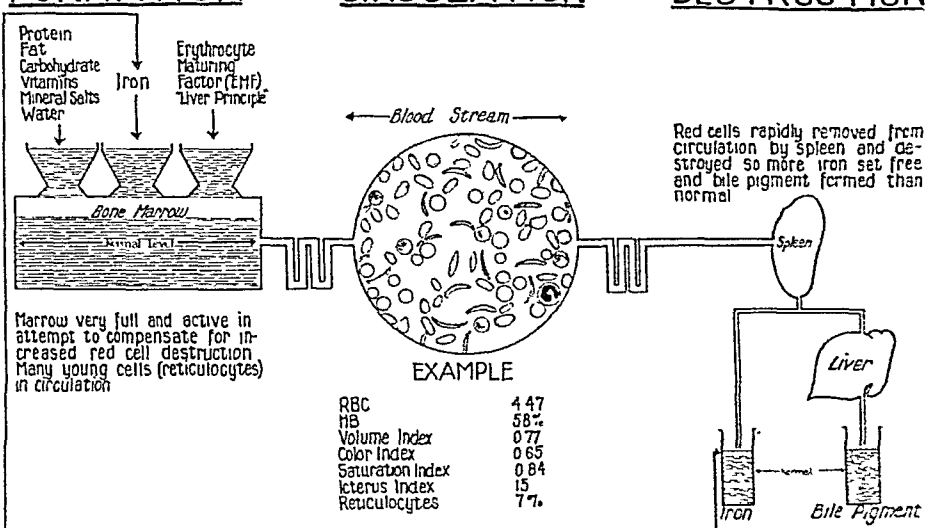


Fig. 9.—Physiology of red cells with excessive filtration by spleen (sickle-cell anemia).

with cells of normal size (volume index, 0.97) and hemoglobin content (color index, 1.02). The reticulocyte count is very low (0.2 per cent). The marrow is at a low level as indicated by the low red cell, white cell, reticulocyte, and platelet counts. The mill is greatly decreased in size although the supply of raw material is ample. There is less destruction of cells so the iron and bile pigment output are much below normal. It is apparent that this anemia can be treated only by measures designed to improve the size and function of the marrow. In this instance the marrow was permanently damaged and the patient finally died of the anemia.

Another type of anemia due to marrow deficiency is illustrated in Fig. 11. This is a myeloid leucemia with a marked anemia (hemoglobin 42 per cent).

TABLE V

## CLINICAL CLASSIFICATION OF ANEMIA

## I Increased blood loss

- 1 Mechanical loss from hemorrhage
- 2 Accelerated red cell destruction by
  - a Hemolytic agents (as phenylhydrazine or bacterial toxin)
  - b Rapid red cell removal from an abnormality of cell shape (as congenital hemolytic icterus), overactivity of reticulo endothelial system, or defect in cell structure

## II Decreased blood formation

- 1 Quantitative decrease in red marrow from aplasia as in benzol poisoning, or crowding out of erythrogenic tissue as in leucemia or myeloma
- 2 Quantitative depression of marrow activity as by malignancy, hypometabolism, chronic toxemia such as nephritis or cirrhosis
- 3 Qualitative decrease in marrow activity from deficiency of specific substances necessary for normal marrow activity
  - a Deficiency in supply, absorption, or use of erythrocyte maturing factor (EMF) as in pernicious anemia or sprue
  - b Deficiency in supply, absorption, or use of iron as in chronic hemorrhage, dietary lack, and idiopathic hypochromic anemia

With a careful study of the blood and determination of each of the indicators of red cell activity, a clinical study of the patient and a clinical classification of the anemia, the different types of anemia can be visualized by means of diagrams. In each diagram, the blood is depicted in relation to the three phases of the red cell, viz, (1) formation, (2) circulation, and (3) destruction. The fundamental fault in the production of the anemia is apparent in such a diagram, so the point of attack in treatment is evident.

In Fig 4, the normal cell is shown in relation to formation, circulation, and destruction. The bone marrow is thought of as a gristmill with three hoppers supplying materials for making red cells. One hopper supplies the nonspecific elements and the other two the specific elements. Normally, the hoppers are full. The level in the mill indicates the relative fullness of the bone marrow. To maintain the normal balance between formation and destruction, nearly one trillion cells and 25 gm hemoglobin must be formed daily. In the circle showing the normal circulation are 100 red cells with one reticulocyte. The cells are of normal size and hemoglobin content. The normal findings are shown below the circle. Old red cells are taken out by the reticulo endothelial cells, largely those of the spleen. If the blood count remains constant as it normally does, the same number must be taken out as are delivered to the blood stream by the marrow. As the hemoglobin is destroyed, iron is split off. Some of the iron is excreted but the larger part (85 per cent) is returned to the marrow to be used again. The end product of hemoglobin destruction is bilirubin which is excreted by the liver. The normal amount of bile pigment and iron formed is indicated by the level of these substances in the containers in which they are received. We think of the mill as functioning at a constant rate of speed so as to supply the same number of cells with the same hemoglobin content as are destroyed each day. The normal mean elapsed time between the beginning of formation of the cell and the ultimate disposal of it is thirty days.

Every anemia can be illustrated by such a diagram. In Fig 5 is shown the red cell mechanism immediately after a large hemorrhage from the uterus.

Here the marrow is full, but the increase in size is due to the hyperplasia of myeloid tissue at the expense of erythrogenic tissue, so there is a great decrease in red-cell-forming tissue and a consequent anemia. The spleen is also enlarged from infiltration of myeloid tissue and not from overactivity due to excessive cell destruction. There is less red cell destruction and so less iron is set free and less bile pigment formed. Here again, the indication for treatment of the anemia is to decrease the mass of myeloid tissue in the marrow by radiation or medication to make room for the erythrogenic tissue. The red cell count often reveals more in leucemia than the number of white cells as it gauges the state of hyperplasia of the marrow which is more important than the white cell count.

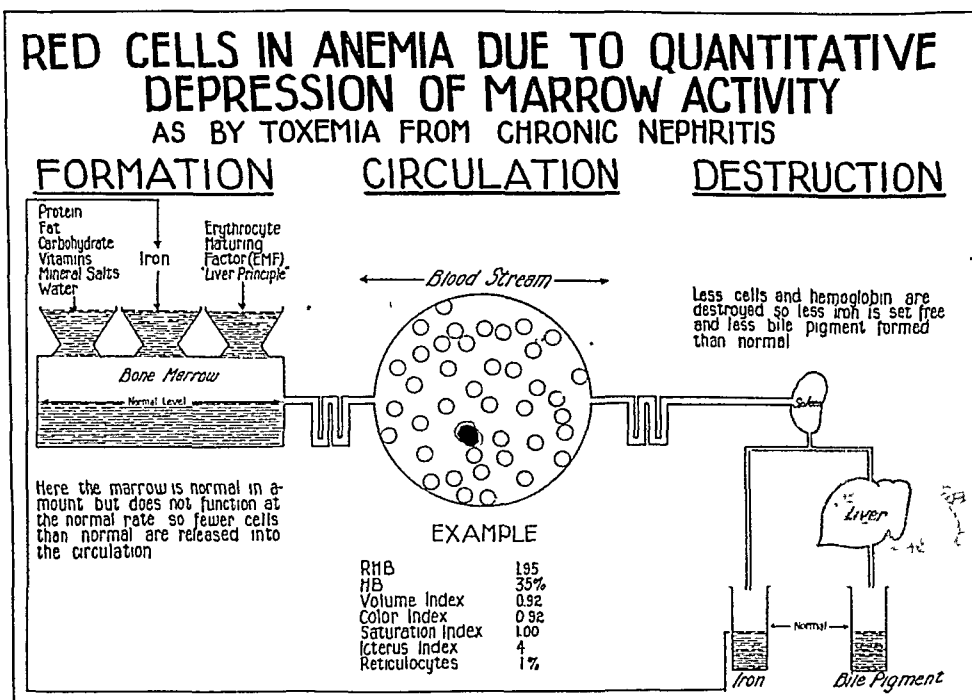
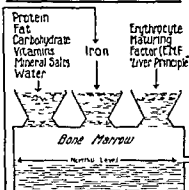


Fig. 12.—Physiology of red cells when the function of marrow is slowed up.

Instead of a quantitative decrease in erythrogenic tissue, the total amount may be unchanged but the function be quantitatively depressed. This type of mechanism is shown in Fig. 12. It is responsible for many cases of anemia such as malignancy, infections, toxemia, and hypometabolism. We can best visualize this mechanism by thinking of it as normal except for the speed with which the apparatus works. It is greatly slowed up, although the supply of building material is normal. Such cells as are turned out are normal and less cells are disposed of. The total number circulating is decreased. The time interval between the beginning of cell formation and the end of cell destruction is increased to varying degrees just as it is decreased in spherocytic anemia or sickle-cell anemia.

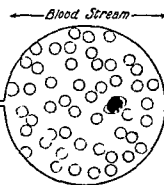
## RED CELLS IN ANEMIA DUE TO ACUTE HEMORRHAGE

### FORMATION



No immediate change in marrow  
Later becomes more active

### CIRCULATION



#### EXAMPLE

RBC	2.50
Hb	50%
Volume Index	100
Color Index	100
Saturation Index	100
Icterus Index	5
Reticulocytes	1%

### DESTRUCTION

Rate of hemoglobin destruction  
is immediately normal. Later it  
is decreased

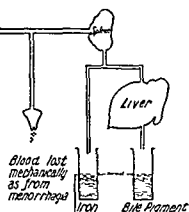
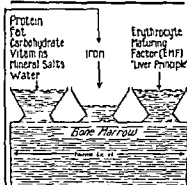


Fig. 5.—Physiology of red cells after an acute hemorrhage.

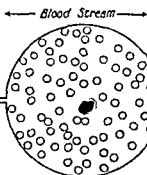
## RED CELLS IN ANEMIA DUE TO CHRONIC HEMORRHAGE

### FORMATION



The bone marrow is hyperplastic  
so fuller than normal in an attempt  
to compensate for the deficiency  
in hemoglobin. The cells released  
into the circulation are smaller  
than normal and have a low  
hemoglobin concentration

### CIRCULATION



#### EXAMPLE

RBC	3.81
Hb	51%
Volume Index	0.67
Color Index	0.45
Saturation Index	0.67
Icterus Index	2
Reticulocytes	0.5%

### DESTRUCTION

There is much less hemoglobin  
destroyed than normal so less  
iron is set free and less bile  
pigment formed than normal

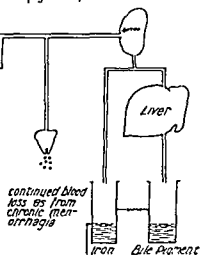
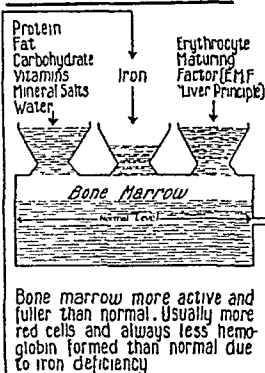


Fig. 6.—Physiology of red cells after a chronic hemorrhage

shown in a chronic hemorrhagic anemia (Fig. 5) except for the mechanical loss of iron in the hemorrhage. In such an instance there is also an iron deficiency anemia due to the loss of iron more rapidly than it is normally supplied by food. If sufficient iron is given, the hemorrhage may continue, but the anemia is relieved so long as the marrow is able to stand the added strain. The bone marrow has to cope with the same deficiency if there is a defect in assimilation so the iron taken in does not reach the marrow. This is the condition in idiopathic hypochromic anemia. In the example cited, insufficient iron has been taken in. The marrow in this instance is hyperplastic in an attempt to compensate, but such red cells as do get out are small

## RED CELLS IN ANEMIA DUE TO DEFICIENT INTAKE OF IRON

### FORMATION



### CIRCULATION

← Blood Stream →

#### EXAMPLE

RBC	40
Hb	50%
Volume Index	0.75
Color Index	0.63
Saturation Index	0.83
Icterus Index	2
Reticulocytes	2%

### DESTRUCTION

Less hemoglobin destroyed  
so less iron set free and less  
bile pigment formed

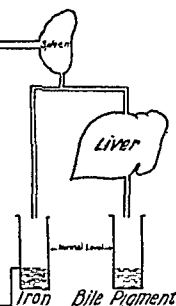


Fig. 14.—Physiology of red cells when building materials are deficient (Iron deficiency anemia).

(volume index, 0.75) and deficient in hemoglobin (color index, 0.67). The low volume and color index indicate the iron deficiency. Here there is much less destruction of hemoglobin so very little iron is set free and the bile pigment content of the plasma is less than normal (icterus index, 2 units). Again the therapy of the anemia is clearly indicated from the diagram. It consists in filling the iron hopper by providing an adequate supply of iron.

If an organ does not receive an adequate supply of a necessary factor, a deficiency necessarily develops. Under certain conditions an organ may receive a necessary factor and for some reason not use it so a deficiency state results just as if the factor were not supplied. The conditions influencing the use of nutritional factors in general have been discussed elsewhere.<sup>7</sup> We may

of building materials is ample so the hoppers are full and the active marrow is fuller than normal. The fundamental difficulty in this disease is an anatomic defect in the shape of the red cells which are spherocytic rather than normal biconcave disks. As a result of this abnormal shape the cells are more fragile than normal<sup>4</sup> and are rapidly removed from the circulation by the spleen which is enlarged as a result of the increased activity. More iron and bilirubin than normal are poured out. Here the average length of life of the red cell is a few days instead of the usual thirty days. There is a rapid stream of cells from the site of origin, the bone marrow, to the place of destruction, the spleen. We cannot correct the anatomic defect so the patient is treated by removing the filter. The abnormally shaped cells function normally if allowed

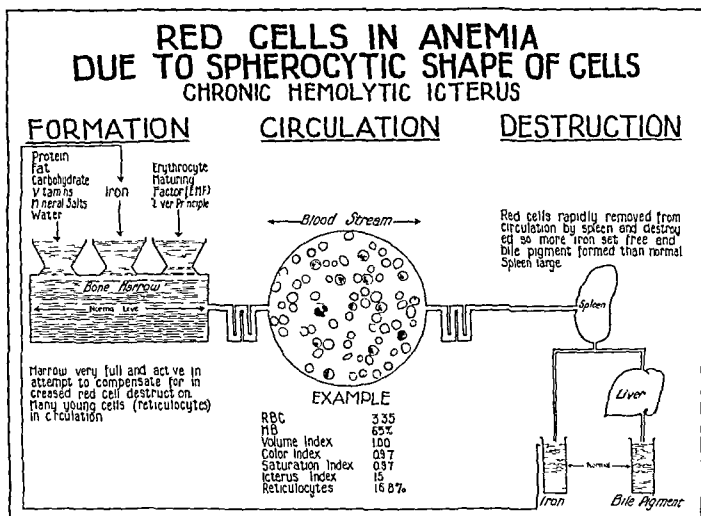


Fig 8—Physiology of red cells with excessive filtration by spleen (spherocytic anemia)

to remain in the circulation. The anemia, reticulocytosis and jaundice all disappear after splenectomy, showing that the increased activity of the spleen is the cause of the anemia although the fundamental defect is in the bone marrow.

Sickle cell anemia, shown in Fig 9, has much in common with spherocytic anemia (congenital hemolytic icterus). Here, also, there is a fundamental defect in the marrow with the delivery of cells of abnormal shape and probably with a greater tendency to fragment. The supply of building material is adequate, the marrow is overactive as shown by the reticulocytosis, and red cell destruction is excessive as shown by the increased icterus index. The spleen is enlarged at least early in the disease due to overactivity in removing

find the clinical and laboratory picture of a deficiency anemia even though an adequate amount of iron and erythrocyte maturing factor be supplied to the marrow.

It is well known that myxedema may show the typical blood picture of pernicious anemia. Such a state is illustrated in Fig. 15. This patient had an anemia with a mild macrocytosis in myxedema. The defect is in the normal completion of the red cells just as it is in idiopathic pernicious anemia. In such a case, the addition of thyroid extract alone should relieve the anemia since the marrow can then use the erythrocyte maturing factor (EMF) already supplied in adequate amounts.

It is quite common to encounter an iron deficiency anemia which will not respond to adequate dosage of iron. There seem to be many more extraneous factors influencing the use of iron by the marrow than of the erythrocyte maturing factor. The example cited in Fig. 16 is the case of a patient with lead poisoning. The giving of iron does not influence the anemia, although the laboratory findings of low volume and color index are characteristic of an iron deficiency anemia. The lead seems to prevent the normal utilization of the iron by the marrow, so treatment must first consist of removing the influencing factor before iron is given. The findings here are exactly like those shown in chronic hemorrhagic anemia (Fig. 6), and an anemia due to a deficient intake of iron (Fig. 14). The laboratory findings indicate the fundamental defect so far as the marrow is concerned but do not show whether the marrow defect is due to excessive loss, deficient intake, or impaired utilization of iron.

#### SUMMARY

In every case of anemia, the rate of red cell formation and delivery from the marrow, the rate of destruction, and the balance between these two factors must be determined.

Measures are available for gauging accurately the state of the marrow and all important factors in red cell activity.

The anemia must be studied and classified from both the clinical and laboratory standpoints.

Red cell formation, circulation, and destruction in all the common anemias are illustrated by diagrams.

#### REFERENCES

1. Barcroft, J.: The Raison d'être of the Red Corpuscle, Harvey Lectures, Series 17, pp. 146-163, 1921-1922.
2. Haden, R. L.: Technic of Blood Examination, J. LAB. & CLIN. MED. 17: 843, 1932.
3. Haden, R. L.: Clinical Significance of Volume and Hemoglobin Content of Red Blood Cell, Arch. Int. Med. 49: 1032, 1932.
4. Haden, R. L.: Mechanism of the Increased Fragility of Erythrocytes in Congenital Hemolytic Jaundice, Am. J. M. Sc. 188: 441, 1934.
5. Landon, J. F., and Patterson, H. A.: Evaluation of Splenectomy in Treatment of Sickle-Cell Anemia, J. Pediat. 7: 472, 1935.
6. Haden, R. L., and Evans, F. D.: Sickle-Cell Anemia in the White Race. Report of Two Cases Benefited by Splenectomy. (Publication pending.)
7. Haden, R. L.: Multiple Specific Nutritional Deficiency Disease in Adult, J. A. M. A. 106: 261, 1936.

# RED CELLS IN ANEMIA DUE TO APLASIA OF MARROW BY MYELOTOKIC AGENT AS ARSPHENAMINE

## FORMATION

## CIRCULATION

## DESTRUCTION

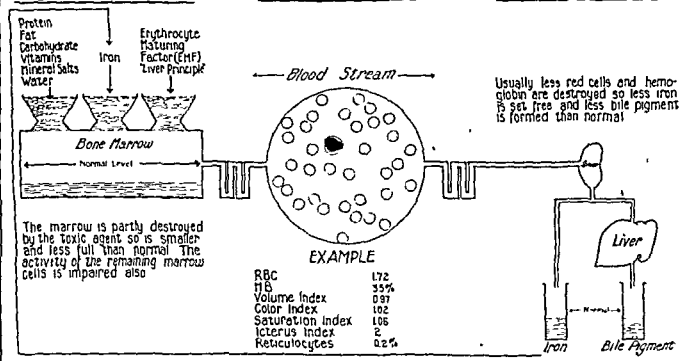


Fig 10—Physiology of red cells when formation of cells is decreased by aplasia of marrow

# RED CELLS IN ANEMIA DUE TO CROWDING OUT OF ERYTHROGENIC TISSUE IN MARROW AS IN LEUKEMIA

## FORMATION

## CIRCULATION

## DESTRUCTION

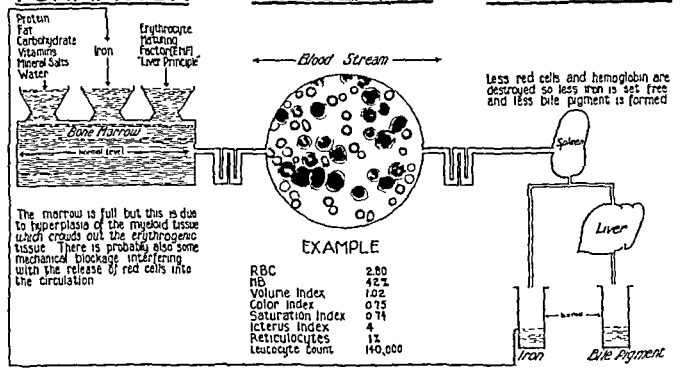


Fig 11—Physiology of red cells where the formation of red cells is diminished by crowding out of erythrogenic tissue

reported positive if treated similarly. We believe that the increase in positive readings is not due to the removal of natural hemolysin but to the introduction of anticomplementary substances adhering to insufficiently washed cells.

As early as 1908, Bauer<sup>2, 3</sup> noted that absorbed serums are increased in anticomplementary properties. Kolmer and Rule<sup>5</sup> have stated that from 2 to 20 per cent of serums may increase in anticomplementary properties following absorption, and this at times may be responsible for the apparent increase in the strength of the reaction rather than to the removal of natural hemolysin. We have found that washing sheep cells as many as five times has not always been sufficient to completely remove the anticomplementary substances that adhere to the cells.

It is possible to devise a titration which will show the reaction which takes place in these serums. Select a serum which contains a large amount of natural hemolysin. Divide it into two parts. Remove the natural hemolysin from one part in the usual way. From the portion which is now free of natural hemolysin, prepare two tubes containing 0.02 c.c. of this serum. To each of these tubes add one unit of complement. To one of the tubes add two units of specific hemolysin, and to the other add one unit. After the addition of the usual amount of 5 per cent cells, incubate both tubes at 37° C. for one-half hour. One tube will hemolyze much slower and much less than the other because of the deficiency of specific hemolysin.

With the original portion of the serum which contains the natural hemolysin, repeat the above procedure. At the completion of this reaction, both tubes will hemolyze to the same extent because the tube which had the one unit of specific hemolysin will be aided by the natural hemolysin present. The tube which received 2 units of specific hemolysin will have such an excess that the additional natural hemolysin will make no difference. If the two tubes which contain one unit of specific hemolysin, one of which contains natural hemolysin and the other does not, are compared, it may be thought that the one which contains natural hemolysin has hemolyzed too much, but this is not so. The fact is that the tube which contains no natural hemolysin has not hemolyzed enough. The reaction in this tube is not complete because of too little hemolysin. This tube is similar to a tube in which the complement and the hemolysin are of equal concentration, and it therefore cannot be used to estimate complement strength. If the other two tubes which contain the two units of specific hemolysin are compared, it will be seen that they are both alike, because both tubes have an excess of hemolysin and the lack of natural hemolysin makes no difference.

These four tubes clearly show that excess of natural hemolysin acts in exactly the same way as excess of specific hemolysin, and it has absolutely no effect on the result of a test except to hasten the end reaction. They also show why natural hemolysin has been accused of producing false negative reactions. It is because too little hemolysin is being used, and those serums which contain natural hemolysin hemolyze in much less time than those which contain none. For this reason, it is assumed that they go too far.

When a slow system is used and the results are read too soon, the only results which are correct and consistent are the negatives. Any positive re-

The anemias due to a defect in supply of building material are most important especially since the lack can readily be supplied. It is in this group that the greatest advances in treatment have been made in recent years. The mechanism of development is now well understood. In Fig. 13 is illustrated the red cell mechanism in an anemia due to a defect in supply to the marrow of the erythrocyte maturing factor (EMF) furnished by the liver and liver substitutes. The anemia I have shown is a typical pernicious anemia. The mechanism is the same in sprue and similar disorders in which the macrocytic anemia occurs. This whole group should be designated the erythrocyte-maturing-factor (EMF) deficiency anemias. Pernicious anemia is the most important of the group. The patient cited during the active phase of the disease

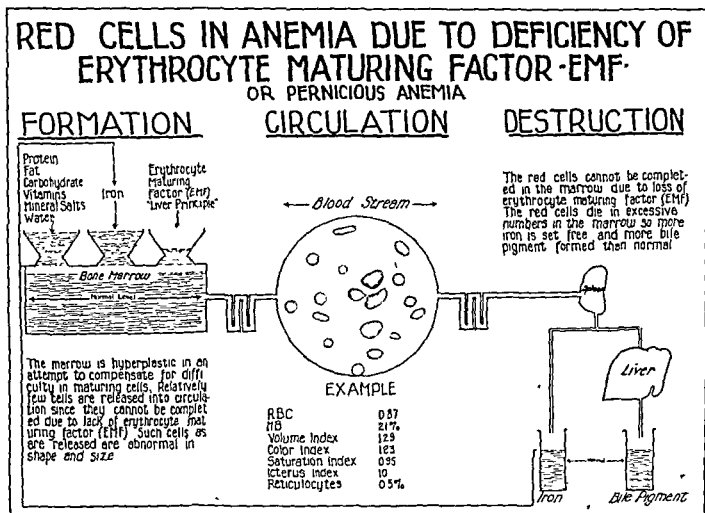


Fig. 13—Physiology of red cells when building materials are deficient (pernicious anemia)

had the macrocytosis of red cells (volume index, 1.29) characteristic of such a deficiency. The marrow in active pernicious anemia is shown by marrow puncture and necropsy studies to be hyperplastic, but very few cells are delivered to the blood stream so the reticulocyte percentage is low (0.5 per cent). The bile pigment of the plasma is high (icterus index, 10 units), indicating an excessive destruction of red cells which in pernicious anemia takes place in the marrow and not in the circulating blood or reticulo-endothelial system. The output of iron is high. Here the therapy is evident. It consists in supplying adequately the deficient erythrocyte maturing factor by giving liver, gastric tissue, or liver concentrates.

The mechanism in an anemia due to a deficient intake or impaired assimilation of iron is shown in Fig. 14. The mechanism is similar to that already

reported positive if treated similarly. We believe that the increase in positive readings is not due to the removal of natural hemolysin but to the introduction of anticomplementary substances adhering to insufficiently washed cells.

As early as 1908, Bauer<sup>2, 3</sup> noted that absorbed serums are increased in anticomplementary properties. Kolmer and Rule<sup>5</sup> have stated that from 2 to 20 per cent of serums may increase in anticomplementary properties following absorption, and this at times may be responsible for the apparent increase in the strength of the reaction rather than to the removal of natural hemolysin. We have found that washing sheep cells as many as five times has not always been sufficient to completely remove the anticomplementary substances that adhere to the cells.

It is possible to devise a titration which will show the reaction which takes place in these serums. Select a serum which contains a large amount of natural hemolysin. Divide it into two parts. Remove the natural hemolysin from one part in the usual way. From the portion which is now free of natural hemolysin, prepare two tubes containing 0.02 c.c. of this serum. To each of these tubes add one unit of complement. To one of the tubes add two units of specific hemolysin, and to the other add one unit. After the addition of the usual amount of 5 per cent cells, incubate both tubes at 37° C. for one-half hour. One tube will hemolyze much slower and much less than the other because of the deficiency of specific hemolysin.

With the original portion of the serum which contains the natural hemolysin, repeat the above procedure. At the completion of this reaction, both tubes will hemolyze to the same extent because the tube which had the one unit of specific hemolysin will be aided by the natural hemolysin present. The tube which received 2 units of specific hemolysin will have such an excess that the additional natural hemolysin will make no difference. If the two tubes which contain one unit of specific hemolysin, one of which contains natural hemolysin and the other does not, are compared, it may be thought that the one which contains natural hemolysin has hemolyzed too much, but this is not so. The fact is that the tube which contains no natural hemolysin has not hemolyzed enough. The reaction in this tube is not complete because of too little hemolysin. This tube is similar to a tube in which the complement and the hemolysin are of equal concentration, and it therefore cannot be used to estimate complement strength. If the other two tubes which contain the two units of specific hemolysin are compared, it will be seen that they are both alike, because both tubes have an excess of hemolysin and the lack of natural hemolysin makes no difference.

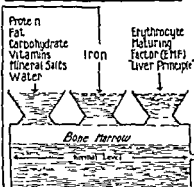
These four tubes clearly show that excess of natural hemolysin acts in exactly the same way as excess of specific hemolysin, and it has absolutely no effect on the result of a test except to hasten the end reaction. They also show why natural hemolysin has been accused of producing false negative reactions. It is because too little hemolysin is being used, and those serums which contain natural hemolysin hemolyze in much less time than those which contain none. For this reason, it is assumed that they go too far.

When a slow system is used and the results are read too soon, the only results which are correct and consistent are the negatives. Any positive re-

# RED CELLS IN ANEMIA DUE TO QUALITATIVE DEPRESSION OF MARROW FUNCTION

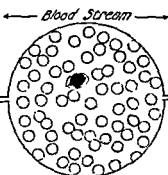
AS IN MYXEDEMA

## FORMATION



In certain cases the bone marrow cannot use the erythrocyte maturing factor (EMF) although present in normal amount. Here the marrow becomes hyperplastic to compensate for the deficiency in cell maturation. Many of the cells released are larger than normal or abnormal in shape.

## CIRCULATION



### EXAMPLE

RBC	314
Hb	61%
Volume Index	106
Color Index	0.91
Saturation Index	0.91
Icterus Index	4
Reticulocyte	1%

## DESTRUCTION

Cells which cannot be matured in the marrow may die in situ in excessive numbers so the iron set free and bile pigment formed may be greater than normal.

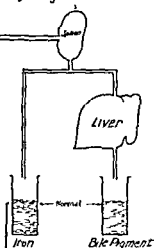
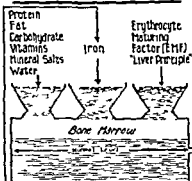


Fig. 15—Physiology of red cells when the marrow is unable to utilize the erythrocyte maturing factor (EMF) normally.

# RED CELLS IN ANEMIA DUE TO QUALITATIVE DEPRESSION OF MARROW ACTIVITY

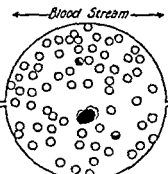
AS IN LEAD POISONING

## FORMATION



In certain cases the utilization of iron in the marrow, although present in normal amounts, is interfered with. Here there is hyperplasia of the marrow to compensate for the insufficiency of hemoglobin. The cells released become small and have a low hemoglobin content.

## CIRCULATION



### EXAMPLE

RBC	330
Hb	46%
Volume Index	0.63
Color Index	0.70
Saturation Index	0.84
Icterus Index	3
Reticulocyte	3%

Less hemoglobin is destroyed so less iron is set free and less bile pigment is formed.

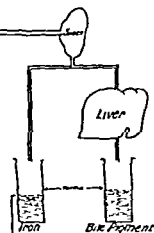


Fig. 16—Physiology of red cells when the marrow is unable to utilize iron normally.

mesenteric capillaries, two contracted (manipulated) endothelial cells seemed to fill the entire channel of a capillary in which there happened to be a physiologic stagnation of flow (v. supra); in no case in which the blood was actually flowing were we able to observe a complete occlusion of the capillary by the localized bulging of the stimulated endothelial cell. With extensive stroking

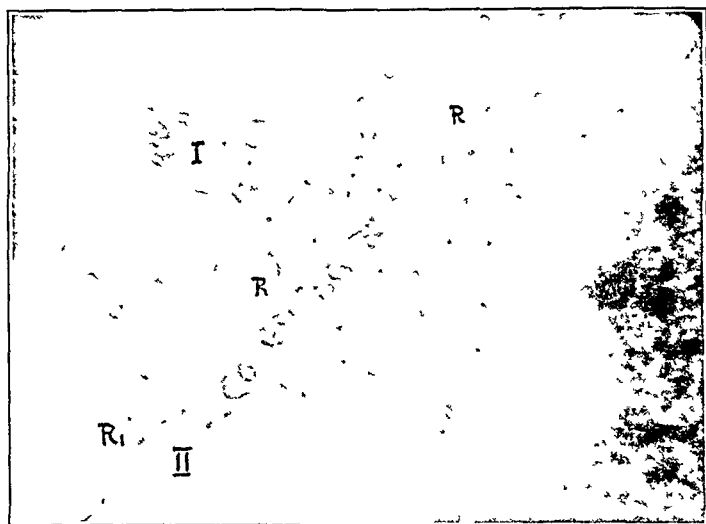


Fig 3—Untouched photomicrograph (frog mesentery), showing (1) spontaneous contraction of capillary (I), (2) generalized contraction of capillary (II), extending from region adjacent to Rouget cell (R) to junction of capillary flow was relieved (in Capillary II) after taking the photomicrograph. The capillary constriction, however, persisted for several minutes.

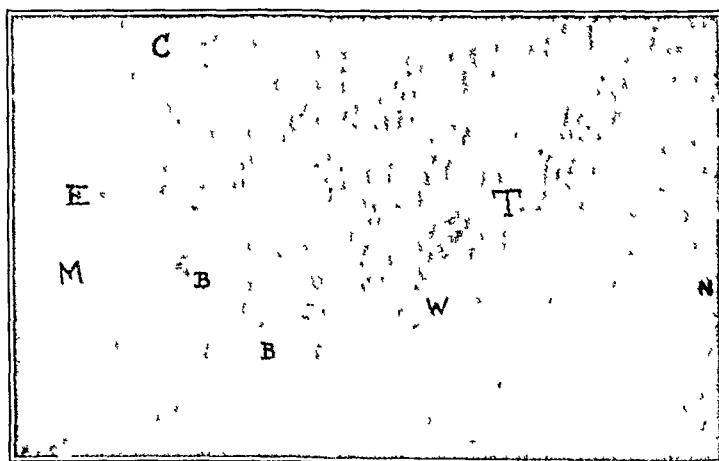


Fig 4—Untouched photomicrograph (frog mesentery), showing (1) maximal capillary dilation and rapid blood flow due to incipient inflammatory reaction, (2) formation of red cell "thrombus" (T) at site of endothelial injury, effected by microdissection needle [a single white corpuscle (W) is attached to lower pole of thrombus] (3) the constricting effect of connective tissue bands (B), (4) a marginating leucocyte (M), and another (L) emigrating through the wall of capillary (C).

of a considerable stretch of the vessel wall it was possible at times to produce a more generalized contraction of the capillary (also arterioles and venules) still without actual arrest of the blood flow (Fig. 3).

# THE QUANTITATIVE RELATIONSHIP OF COMPLEMENT TO HEMOLYSIN ITS DIRECT APPLICATION IN THE SERODIAGNOSIS OF SYPHILIS\*

JOHN KOOPMAN, AND I DAVID FALKER, NEW YORK, N Y

THE quantitative relationship of complement to hemolysin is of more than academic importance. It has a direct bearing upon the accuracy and consistency of all complement fixation tests. It is also the fundamental factor needed to solve the problem of the effect of the natural hemolysin in patients' serums on complement fixation results.

The experiments outlined in this paper demonstrate the fact that in complement fixation reactions an excess of one reagent does not compensate for a deficiency of the other, and any method which is based upon the theory of compensation cannot produce reliable or consistent results.

A titration which is useful in disclosing the relationship existing between complement and hemolysin is shown in Table I.

This titration is planned so that various amounts of complement from a minimum (0.01 cc) to a maximum (0.1 cc) are allowed to act with various amounts of hemolysin from a minimum to a maximum. The effect desired will be more clearly shown if the complement and hemolysin are diluted, so that each solution contains one unit in a volume of 0.1 cc. The cells are a 5 per cent suspension and 0.1 cc is used in each tube. The total volume is 0.5 cc. This titration should be allowed to remain in the water bath at 37° C. for at least one hour.

TABLE I\*

HEMOLYSIN										COMPLEMENT
0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	
0	0	0	0	0	0	0	0	0	0	0.01
0	0	0	0	1	2	2	2	2	2	0.02
0	0	0	1	2	2	3	3	3	3	0.03
0	0	1	2	2	3	3	4	4	4	0.04
0	0	1	2	2	3	3	4	5	5	0.05
0	1	2	2	2	3	4	5	6	6	0.06
0	2	2			4	4	5	6	7	0.07
0	2		4	4	4	5	6	7	8	0.08
0	2		4	5	5	6	7	8	9	0.09
0	2	3	4	5	6	7	8	9	10	0.1

\*0 No hemolysis

10 Complete hemolysis

Assigning arbitrary figures to the amount of hemolysis obtained from zero up to 10, which represents complete hemolysis, it will be seen that 0.01 cc of complement does not produce any hemolysis, regardless of the amount of hemolysin used. With 0.02 cc of complement the first trace of hemolysis appears with

\*From the Wassermann Laboratory of the Department of Health.  
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of platelets and leucocytes, the so-called "white thrombus" (Zahn). The coagulative changes (fibrin formation) in the blood plasma are indifferently demonstrable with the present technic.

We take pleasure in acknowledging the helpful advice of Prof. J. F. Fulton during a large part of these observations, which were made in the laboratory of Physiology, School of Medicine, Yale University.

#### REFERENCES

1. Clark, E. R., and Clark, E. L.: The Relation of "Rouget" Cells to Capillary Contractibility, *Am. J. Anat.* 35: 265, 1925.
2. Clark, E. R., and Clark, E. L.: Observations on Living Preformed Blood Vessels as Seen in a Transparent Chamber Inserted Into the Rabbit's Ear, *Am. J. Anat.* 49: 441, 1932.
3. Zweifach, B. W.: Micromanipulative Study of Blood Capillaries, *Anat. Rec.* 59: 83, 1934.

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## VISCERAL TEMPERATURES IN THE INTACT AND UNANESTHETIZED ANIMAL\*

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### I. A NEW TECHNIC FOR MEASUREMENT

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THE temperatures of the internal organs of the animal body and their relationship have never been comprehensively studied, although there have been numerous reports concerning the degree of heat possessed by one or two organs under specialized conditions, as for example, the stomach after food administration. This literature, however, presents variant and confusing figures for organic temperatures both in experimental and in normal states. The confusion has largely arisen from certain inaccuracies commonly inscribed in these reports, of which the most frequent are: (1) exposure of the viscera to the cooler air of the experimental chamber, (2) changes in body temperature, blood flow, and heat regulation due to anesthesia, (3) variability in the standardization of a base-line body temperature, and (4) instrumental errors. A brief consideration reveals the magnitude of these errors, which are corrected by the new technic.

1. *Exposure*.—Fig. 1 represents the uniformity of individual organic and general body temperatures obtained with the technic reported in this paper, which eliminates the effects of exposure by permitting forty-eight hours to elapse between the operative placement of the thermometric device on the organ and the measurement of temperature. The technical errors necessarily introduced by the older technics, which involved measurement immediately upon application of a thermometer or other instrument, are seen by the facility with which this organ-body uniformity of temperature is destroyed. When an incision is made through the skin alone, the organs underlying the opening

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\*From the Department of Anatomy, Yale Medical School.

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This work was done in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Yale University.

actions obtained with a slow system are subject to criticism unless hemolysis is allowed to proceed for so long a time that one may be certain that the reaction is finished

It is well known that a small percentage of specimens from cases of clinical syphilis fail to give positive reactions. It is quite convenient to explain failure to secure a positive result in such cases by pointing to the natural hemolysin. If this natural hemolysin is removed and the reaction considerably delayed, as it would be with a slow system, the reaction may be read too soon and therefore read too strong. Notwithstanding the fact that some of these specimens are clinically positive, they are nevertheless false positives as far as the Wassermann reaction is concerned, if the result is made positive by the removal of natural hemolysin.

When the proper amount of specific hemolysin is used, the test is sensitive and the end point is sharp. If under such circumstances a clinical comparison shows that too few positives are obtained, the remedy is to use less complement and to read the results when the reaction is complete. Any attempt to obtain more positives by reducing the hemolysin will produce unreliable results.

#### CONCLUSIONS

There is no danger of complement fixation tests hemolyzing too fast nor too far when an excess of hemolysin is used.

There is nothing to be gained by the removal of natural hemolysin from patients' serums. On the contrary, false positives may occasionally be obtained.

*An excess of hemolysin does not compensate for a lack of complement.*

*An excess of complement does not compensate for a lack of hemolysin.*

#### REFERENCES

- 1 Bailey, C. H. The Value of Absorption Methods in Wassermann Tests, *Arch. Int. Med.* 9: 551, 1912.
- 2 Bauer, J. Zur Methodik des serologischen Luesnachweises, *Deutsche med. Wchnschr.* 34: 698, 1908.
- 3 Bauer, J. Zur Wesen der Wassermannschen Luesreaktion. *Berl. klin. Wchnschr.* 65: 834, 1908.
- 4 Jacobaeus, H. C. Die storende Einwirkung der im Menschen Serum enthaltenen natürlichen Ambozeptoren bei Wassermanns Reaktion, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 8: 615, 1911.
- 5 Kolmer, J. A. and Rule, A. The Influence of Natural Anti Sheep Hemolysin in Human Serum Upon the Wassermann Reaction. *Am. J. Syph.* 4: 135, 1920.
- 6 Koopman, J., and Falker, I. D. The Inadequacy of Present Complement Titrations, *J. Lab. & Clin. Med.* 21: 312, 1935.
- 7 Morgenroth, J. F., and Sachs, H. *Gesammelte Arbeiten für Immunitätsforschung*, Berlin, 1904, Hirschwald.
- 8 Olmstead, M. P. Value of Absorption Methods in the Wassermann Test, *Med. Rec.* 85: 341, 1914.
- 9 Ottenberg, R. On the Reliability of the Wassermann Reaction, *Arch. Int. Med.* 19: 467, 1917.
- 10 Rossi, O. Ueber die Methodik der Wassermannschen Syphilis reaction. Ein Verfahren zwecks Absorption der Menschen Serum Normalerweise enthaltenen Ambozeptoren gegen rote Hammalblutkörperchen, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 10: 321, 1911.
- 11 Simon, C. E. The So called Doubtful or Partial Wassermann Reactions, *J. A. M. A.* 72: 1535, 1917.

correlated peripheral vasodilatation, which in a hot room does not produce the ordinary thermolysis, but the converse, a progressive degree of heat soon to end in heat-stroke if the air temperature is not lowered.

3. *Variability in Standardization of a Base-Line Body Temperature.*—Uniformity of depth and a sufficiently long latent period for the thermometric device to assume the environmental degree of warmth are inviolate precepts in measurement of body temperature in rectum or vagina. A consideration of Fig. 3 illustrates polythermocouple recordings of variant degrees in the same animal which were obtained at rectal depths of 0.6 cm., 1.8 cm., and

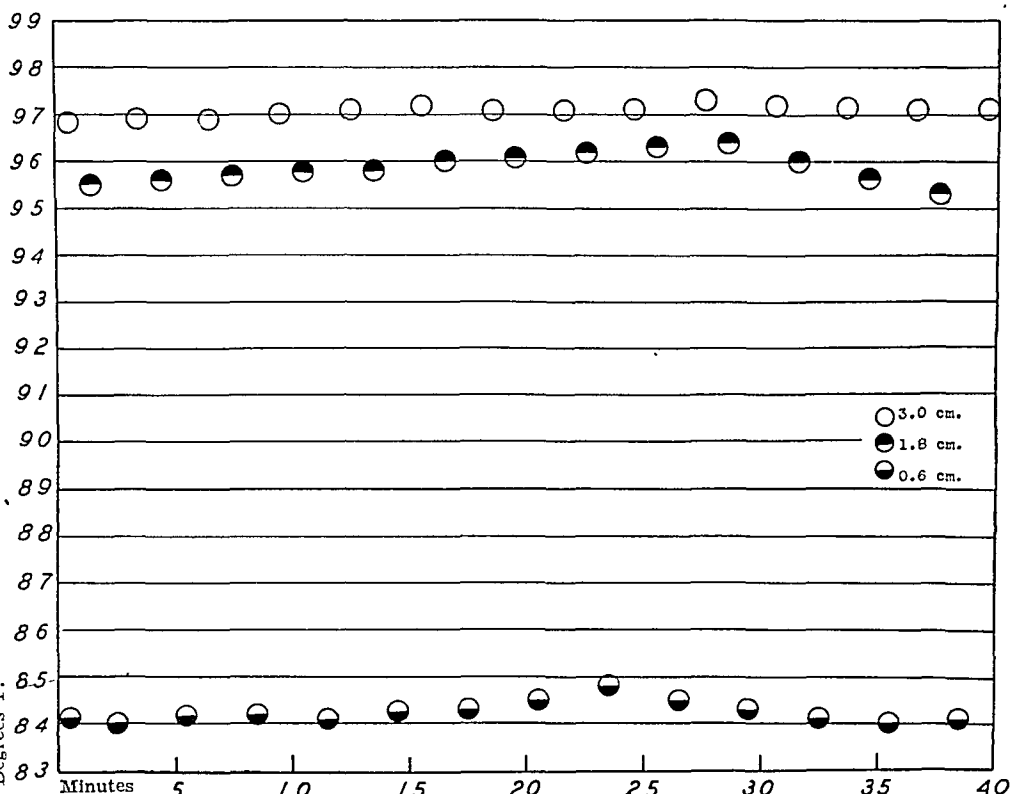


Fig. 3.—Gradient of rectal temperature of the rat at depths of 3.0 cm., 1.8 cm., and 0.6 cm.

3.0 cm. The depth of insertion necessary before approximation of the “internal abdominal temperature” differs among the various animals.

4. *Instrumental Errors.*—Instrumental errors are liable to be many and varied and do not warrant separate discussion in this place. Some errors to which simple galvanometer circuits are subject can be avoided by the use of a “null circuit,” wherein no current flows through the apparatus.

Thus it can be seen that visceral exposure and disturbances of heat regulation are incident to anesthesia and operative insertion of devices for thermal measurement, and result in after-effects lasting as long as twenty-four hours in which organic and body heat alike are abnormal. Normal thermal regula-

stained the cytoplasm of the pericytes and differentiated those of tissue origin from emigrated leucocytes. Such *leucocytes* could also be distinguished by rounding off more completely when stimulated with the microneedle. In addition, they were easily detached. The true pericytes, on the other hand, assumed an oval form (see Fig. 2) and often resisted detachment from the vessel wall. Micromanipulation of the *endothelial cells*, a single nucleus usually being selected

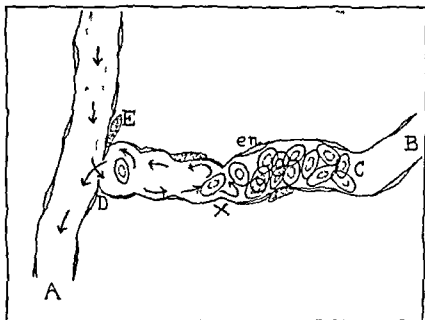


FIG. 1—Sketch of eddying of blood into mouth of patent capillary during phase of "physiologic stagnation", showing (A) parent vessel, (B) capillary, (C) stagnating mass of blood corpuscles, (D) constricted "mouth" of capillary, (E) Rouget cell, (en) endothelial cell nucleus (X) (see text)

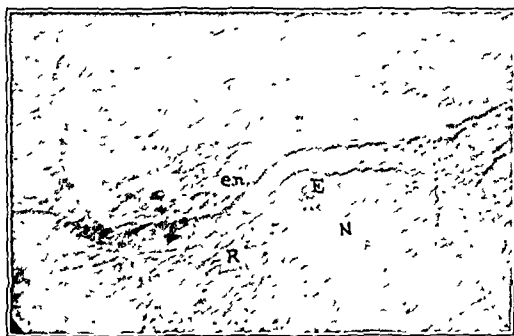


FIG. 2—Untouched photomicrograph, showing localized contraction of single endothelial cell (E) of frog's mesenteric capillary, in response to micromanipulation by needle (N). The appearance prior to stimulation was very similar to (en). A previously manipulated Rouget cell (R) may be drawn slightly from the capillary wall without affecting the underlying blood vessel. There is "physiologic" flow unrelated to the manipulative procedures.

for stimulation, resulted (in a fresh preparation) in the cell-nucleus becoming swollen and ovoid. At the same time, or shortly thereafter, the cytoplasm shortened longitudinally and bulged transversely, producing a localized narrowing of the capillary lumen. Although in one experiment with the kitten's

precautions are taken to prevent inaccuracies. Neither jarring of the instrument nor elevation of the glass lid is permitted during readings; the paper roll is prevented from sliding. Further, the galvanometer is adjusted before each test to avoid machine "drift" and calibrated against a known temperature at the beginning and end of each experiment.\*

The micromax is allowed a period of an hour for self-adjustment before readings are begun. Where leads record divergent temperatures, as in concomitant skin and rectal determinations, each lead is run solely on itself for a five-minute period to ascertain the correct degree for that particular lead, irrespective of any deviation due to divergence of the other leads. By means of these precautions the instrumental error is always less than  $0.1^{\circ}\text{F}$ ., when compared with the known bath temperatures. There is no need, however, of claiming for these experiments an accuracy greater than the manufacturer's estimated error of  $0.9^{\circ}\text{F}$ . The probability that such a large error exists in

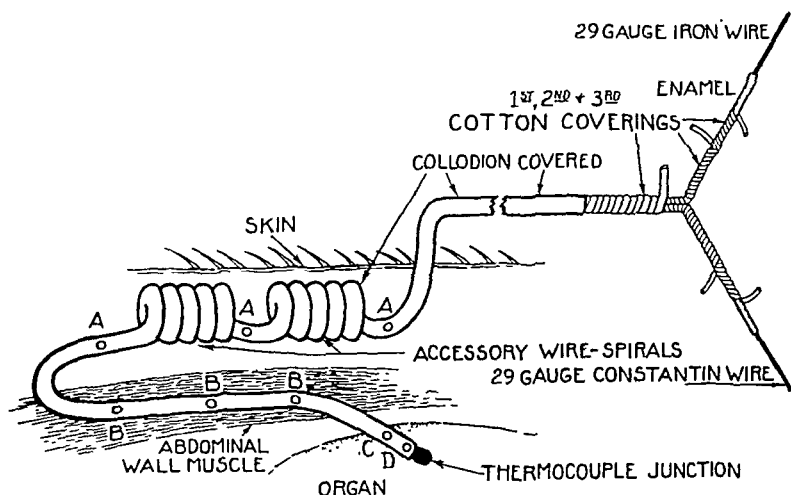


Fig. 4.—Mode of attachment and type of thermocouple employed in temperature measurements.

spite of these precautions is slight, as seen by the constancy obtained in the measurement of internal organs. Fig. 1 shows uterine and rectal temperatures of a rat, which vary less than  $0.02^{\circ}\text{F}$ ., from one another over the half-hour test period, despite the accentuation of any machine error by animal fluctuations!

**Thermocouples.**—The thermocouples are of fine 29 gauge wire, iron and constantin being selected in view of the type of wiring in the recording potentiometer. Each wire is enamelled and then wound separately with two thread windings, the threads running in opposite directions (Fig. 4). A third layer of thread binds together the separately covered wires, the whole then receiving a coat of collodion before implantation.

\*For this purpose sodium sulphate is kept at the transition point ( $90.3^{\circ}\text{F}$ .) by a vacuum bottle enclosed in heat-insulating cellox, the whole boxed with wood. The heat of the sulphate, which is practically constant over a period of twelve hours, is registered by a Beckmann differential thermometer calibrated to  $0.025^{\circ}\text{F}$ . The micromax records against the known temperature of the sulphate in the vacuum bottle by a thermocouple placed 0.5 cm. from the thermometer. The difference between this reading and that of the sulphate constitutes the error which can be calculated with reference to points on the curve of the experimental readings.

*Experimental Capillary Injury in the Frog's Mesentery.*—By sufficiently vigorous movements of the microdissection needle, especially in capillaries dilated by reason of early inflammatory reaction, it was easy to injure the endothelial lining with the result that first one, and then more, passing blood corpuscles began to adhere to the injured site and to each other to form a "thrombus." It was significant that the colorless corpuscles (from which the thrombocytes could not be differentiated) evinced a greater *stickiness* toward the injured epithelium. Nevertheless, the more numerous red cells usually played the chief rôle in the thrombus formation. Only if the capillary was widely dilated and the blood flow swift did the red cells keep getting pulled off the injured region by the force of the blood stream, and the thrombus formation in these cases became permanent only when the more firmly adhering white corpuscles chanced to contact the damaged endothelium. A pure red cell thrombus is seen in Fig. 4.

#### DISCUSSION

*Capillary Contractility.*—The evidence of direct mechanical stimulation, strictly localized by micromanipulation, is conclusively in favor of an independent contractility of the capillary endothelial cells (confirming Zweifach).<sup>3</sup> While pericytes (including Rouget cells) are also contractile, in common with many other cell types, the evidence is all against their contraction in any essential manner affecting the caliber of the underlying capillaries. It is a varicosity, produced by the localized restricting effects of unyielding connective tissue fibrils or firmly anchored pericytes, which gives the appearance of localized constrictions in otherwise dilated capillaries.

*Regulation of Capillary Flow.*—A frequent absence of correlation between blood flow and capillary diameter was noted, with the two extremes, viz. (a) vigorous efforts at circulation through generalized or localized capillary constrictions and (b) abeyance ("physiologic stagnation") of blood flow in demonstrably patent, and perhaps well-dilated, capillaries. The data suggest (in confirmation of Clark and Clark<sup>1</sup>) that blood pressure differences between the two ends of the capillary play the major rôle in regulating the blood flow through each particular capillary. Of course, it is entirely possible that variations in the capillary caliber assist in redistributing these "pressure differences" and that the actual amount of blood flow in a unit of time is a direct function of the capillary diameter.

*Mechanism of Capillary Thrombosis.*—The present experimental data help to clarify the understanding of variations in the types of thrombus formation, already well recognized to bear a relationship to the degree of stagnation of the blood flow. Our experiments indicate that, with a slow rate of blood flow, the corpuscular elements adhere indiscriminately to the damaged vessel wall and tend to form a thrombus consisting of the most numerous, viz. the red cells. This is recognized to be the type which ultimately forms the so-called "hyaline thrombus" (von Recklinghausen). With a more rapid blood flow, however, the red cells are unable to adhere sufficiently firmly to resist being swept on by the blood stream, and the thrombus finally formed is, therefore, made up chiefly

an attachment mechanically prevents the blood from flowing through the tissue. Since the electric current of the circuit is a resultant of the heat over the entire surface of the junction, that part of the junction not in contact with the skin is insulated with an enamel coating. The practice commonly employed of placing the thermocouple under adhesive tape on the skin prevents proper radiation and convection at the surface. The question of a stable skin-junction attachment is further complicated by slight movements of the animal which tend to dislodge the thermocouple. To overcome these difficulties, the thermocouple is suspended by twine in the center of a ring of bone so as to bear only slightly yet continuously against the skin. Where taping the ring to the foot or ankle of a small animal would mean occlusion of the blood flow, the alternative of attaching the ring to the foot is accomplished by stabilizing the foot on the ring.

*Immobilization Cages and Halters.*—Where cooperation cannot be expected from the subject, there arises the problem of obtaining forced acquiescence in as quiet a manner as possible. For this purpose comfortable mesh-wire cages with detachable top and back are designed to fit each animal snugly enough to eliminate movements. Fig. 5 shows the assembled cage containing an immobilized animal. The top-piece moves up or down to accommodate the individual animal. Pinions through the meshes of the cage and the movable top-piece hold the latter in place. The backpiece is provided with an open center through which extends the tail of the animal. Thus, the rectal thermocouple is readily inserted to the correct depth, then taped to the tail to prevent change in its position. The rearpiece is also held in place by pinions through it and the cage. The mesh wire provides unimpeded air contact with the surface of the animal for radiation, convection, and evaporation.

The rodent habit of continual gnawing is directed at the thermocouple with the result that the animal often bites through the wire at the surface of the skin, thus destroying the possibility of connection with the machine. A satisfactory means of eliminating wire-gnawing is devised by a system of halters. Light copper tubing, 0.4 cm. in diameter, is bent in pairs of omega-shaped pieces fitted together to form an adjustable collar by a bolt and nut at each end of the half-circles. The animals readily feed and clean themselves but are prevented by the tubing from reaching the region where the implanted wires leave the body. These light though durable halters can be worn at all times. The cylindrical form of the rat permits turning in a cage just loose enough to avoid cramping, but temporary attachment of the halter to the immobilization cage prevents the animal from twisting about. Revolutions within the cage are avoided, for the machine-connected thermocouple wires allow but slight movement, as turning pulls heavily on the protruding wire when stabilized by attachment to the machine lead.

The advantages of this method of restraint may be listed as follows:

1. *Limitation of Animal Movements.*—Retention of the rectal thermocouple throughout the test period, during which the animal is immobilized, minimizes the excitement and motion that arise in the older methods of thermometer insertion and handling at the time of measurement. That the higher temperature at the beginning of the immobilization is not due solely to the excitement

in the skin are cooled, although the thick layer of abdominal muscles remains intact (Fig 2) These effects of abdominal exposure may endure for considerable time, with only partial recovery of normal thermal uniformity as long as twenty four hours after operative exposure Thus it can be seen that the older methods of acute experiments do not adequately protect temperature determinations from inaccuracies introduced by exposure

2 *Anesthesia*—As early as 1896 Allen<sup>1</sup> realized that the body heat level might be either raised or lowered during anesthesia, the direction depending upon the environment Since a return to the pre anesthetic body level is a variable process, experiments purposing to measure normal organic temperatures need allow sufficient time for proper recovery It is conceivable, how

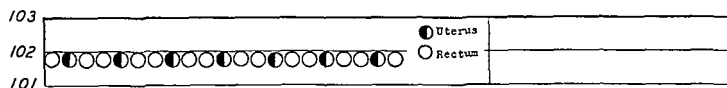


Fig 1—Uniformity of uterine and general body temperature in the unanesthetized rat

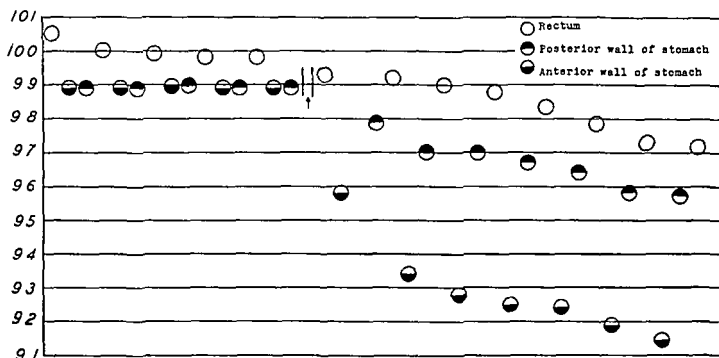


Fig 2—Destruction of uniformity of abdominal temperatures after visceral exposure by an unclosed epigastric incision 0.7 cm in length through the muscles of the abdominal wall. Arrow denotes elapse of five minutes after separation of the wound edges with forceps. Animal a 320 gm rat

ever, that body warmth may be maintained by insulative wrappings around the animal plus a sufficiently high room temperature, notwithstanding, the animal is not physiologically normal, in view of the derangement of thermal regulatory centers in the body and the abandonment of the normally existent thermal gradients between viscera and the environment

It is further necessary to understand the difficulties confronting any attempt to maintain the normal degree of heat in *exposed* viscera by thermal adjustment of the outside air To prevent exchange of heat, air and organs would of a necessity be thermally identical, which would mean a room warmth approximating 100° F delicately adjustable to slight variations in animal heat But the incision for the measuring device requires an anesthesia with

surrounding them. The individual organs are also affected when the general temperature of the animal is elevated. The colon is easily accessible without operative implantation of thermocouples, yet sheltered from direct reaction to environmental change. The depth of insertion was standardized at 7.5 cm. in rats weighing over 150 gm., and 2.5 cm. in the mouse; in larger animals such as the cat, dog, and monkey, 12 cm. was used.

Rats were given a two weeks' period of training, during which daily vaginal smears were recorded for all females. During the first days of training the rats struggled violently to escape from the cage; therefore, temperature measurements which were taken throughout the training period were high for the first days. They soon became accustomed to the two-hour period of daily immobilization, sometimes choosing to return to the cage after liberation. The intense excitement of the early training was replaced by a quiescent behavior which promoted a rather uniform level of body heat. Slight movements were never entirely eliminated; these, however, did not cause increase in body temperature. Most of this motion was in the form of intermittent gnawing on the metal cage, together with small shifts of body posture every few minutes, much in the manner of any sleeping animal.

No difficulty was experienced in getting the animals to enter the cages. Occasionally a rat dallied about for a moment, but to overcome this reluctance without exciting the animal, the experimenter had only to hold the rat's head in the direction of the cage opening, whereupon it entered of its own accord. The small quarters of the cage seemed to cramp the animals but little, for they ran limberly about upon release after a two-hour confinement. When the tests extended over long periods, as in forty-eight-hour tests of daily rhythm, the top-piece was lifted off at about four-hour intervals to permit stretching and radical changing in the animal's position.

At the end of the two weeks' training period those rats were selected for experimentation which had shown a stable body temperature, a cessation of the tendency to struggle upon confinement in the cage, and in the females a four- to seven-day estrual rhythm.

Operations were performed upon ether anesthesia with aseptic precautions. Special sutures of twenty-day chromic catgut, 0000, with half-circle atraumatic needles were used.\* A suture through a loop in the thermocouple (*D* in Fig. 4) was lightly sewn to the mesentery of the organ. By a loop (*C* in Fig. 4) farther from the measuring junction, the wire was firmly sutured to the muscular wall or capsule of the organ. Duplicate measurements were made from thermocouples implanted within the organ. From the organ the wire pierced the abdominal muscle to pass beneath the skin for a distance of 4 cm. before penetrating to the surface. This procedure plus a tight closure of muscle and skin served to prevent abdominal exposure along the passageway of the wire. The thermocouple was attached to the muscle of the abdominal wall and to the overlying skin (loops *B* and *A* respectively in Fig. 4).

Where possible the wires were led out through the skin near the midline at a level with the last rib. This minimized gnawing of the wire, and in addition,

\*These sutures were specially prepared through the courtesy of Davis and Geck, Inc.

tion and body heat levels are liable to perversion in the most carefully conducted acute experiments. The older work is without exception composed of acute experiments in which measurements were conducted immediately upon placement of the measuring device or shortly after closure of the incision.

Hence, because of these errors in measurement, the one point of agreement among previous workers has been that the internal organs have been found to differ in their degree of heat. In general, those organs established as warmer have been either (1) the more vascular, therefore more able to resist exposure, as the liver, or (2) the more protected from exposure during examination, as the underside of the stomach. Differential organic temperatures have resulted from variance in ability to resist exposure and disturbances in bodily heat regulation.

#### PROCEDURE

In the present experiments a technic has been devised to minimize the inaccuracies entering into temperature determinations, and to permit investigation of the intact animal without anesthesia. A fine thermocouple is implanted upon the organ to be tested, and the animal allowed forty eight hours before measurement, this length of time was found sufficient for recovery from visceral exposure and anesthesia. Standardized body temperatures are obtained by training animals to lie quietly with a thermocouple introduced through the anus for continuous measurement.

*Apparatus*—A Leeds Northrup multiple point recorder, trade named "Micromax," is suitable for most measurements. This machine records temperatures by determining the E M F necessary to equal the E M F of the thermocouple circuit. No current flows through the galvanometer, thus preventing errors which accrue in a simple galvanometer circuit upon the following changes in the thermocouple circuit:

1. Variations of resistance in the thermocouple circuit, including changes due to the employment of different lengths of wire. This is a crucial consideration, since a substitution of wire is necessary if the wire snaps in the course of lengthy experiments. Advantage is further taken of the unimportance of resistance variations by embedding additional lengths of wire beneath the animal's skin to be used in the case of occasional breaks or inadvertent gnawing by the animal.

2. Passage of the leads through areas differing in temperature. A practical test made by recording against a constant temperature bath while the temperatures of the two wires are varied as much as 100° F., proves that variation in the warmth of the thermocouple connections does not influence the readings.

The micromax possesses an automatic cold junction compensator and a self adjusting potentiometer circuit, which standardizes itself at every thirtieth reading interval. Thus, one investigator can obtain readings more accurately over longer periods of time than would be possible for several workers using a simple galvanometer.

The limitations of the machine are significant although not entirely evident from superficial appraisal. Nondiscerning manipulation may introduce errors of 2° F. To determine the practical accuracy under the conditions of the experimentation, the readings used in this work are checked against a constant temperature bath which is capable of regulation of 0.03° F. Other

diathermy, radiothermy, radiant heat and other general or local applications. Implanted thermocouples provide an adequate experimental means for exact determination of the depth of heat penetration, the spread through surrounding tissues, the duration of the after-effects, and the most effective dosages. Thermal therapy would be greatly benefited by such exact data.

#### SUMMARY

1. The problems inherent in the measurement of visceral temperatures are discussed with regard to the inaccuracies introduced heretofore. A new technic is described for temperature determination in intact and unanesthetized animals, which corrects the following errors found in the older method of acute experiments:

a. Exposure of the viscera to the air of the experimental chamber either shortly before or at the time of temperature determination. As long as forty-eight hours may be required for complete recovery from exposure.

b. Changes in body temperature, blood flow, and heat regulation due to anesthesia.

c. Variability in the standardization of a base-line body temperature.

d. Instrumental errors.

Particulars are given of the apparatus, operative placement of the thermocouples, and the selection, training, and care of the animals.

2. A method is described for training and immobilization of animals to eliminate excitement, movement, and handling incident to ordinary rectal measurement by thermometers, and to permit continuous determination of temperature over long periods of time (100 hours).

3. Temperature measurements by means of this new technic of implanted thermocouples give no evidence for the claims advanced in the literature of differences in the degree of heat in the various abdominal organs. The uniformity of internal body temperatures will be discussed in a series of investigations of visceral temperature in various types of animals.

4. The technic is of value in determining exactly the internal and subdermal temperatures with reference to problems in therapeutic heating, thermal gradients from viscera to periphery, and nervous and glandular functions.

#### REFERENCES

1. Allen, D.: *Trans. Am. Surg. Assn.* 14: 367, 1896.
2. Harvey, S.: *Arch. Surg.* 18: 1227, 1929.
3. Bazett, H. C., and McGlone, B.: *J. LAB. & CLIN. MED.* 76: 89, 1926; *Skand. Arch. f. Physiol.* 48: 89, 1926; *J. LAB. & CLIN. MED.* 82: 415, 1927.
4. Foster, P. C.: *Proc. Soc. Exper. Biol. & Med.* 33: 62, 1935.

Care is taken to limit to 10 mm the size of the junction imbedded in the animal. After removal of traces of solder resin, the wires are reinsulated up to the exact point of junction. Circular loops (*C* and *D* in Fig. 4) in each wire are a means of sutural attachment to the organ.

In preliminary experiments the thermocouple wire was of sufficient length to extend from the animal to the galvanometer. When not in use, it was wound around a small piece of cardboard which could be fastened to a small jacket worn by the animal. During the course of the experiments, it was found more expedient to extend the leads from the machine to the animal; thus, only 2 cm. of wire project outside of the skin. A secure, yet quickly adjustable connection between machine and animal wires is procured by means of binding posts similar to the type used to fasten detachable antenna wires to radio sets. One end of the binding post is soldered permanently to the machine lead; the other end, a screw nut with a slot to connect with animal

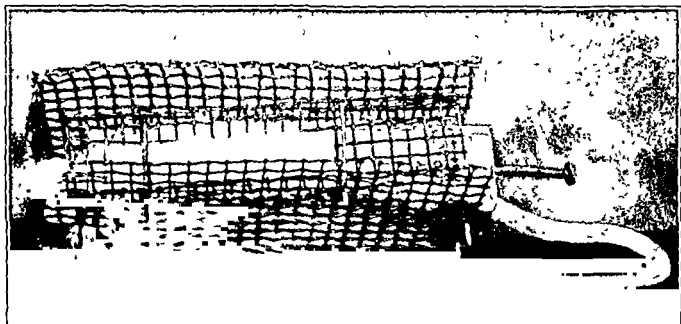


Fig. 5—Simple adjustable immobilization cage in which animals may be trained to rest quietly

leads, can be changed from animal to animal. The posts are insulated from the cage and one another by hard rubber.

The rectal (actually "colonic" in small animals) thermocouple is cemented within a flexible, hard catheter, size 9. Flexibility is essential if the thermocouple is to be kept in the animal over any extended period, or if tests are run from day to day, as it must be adaptable to movements of the animal. The smoothly rounded surface of the thermocouple junction, extending 2 mm. beyond the open end of the catheter, is firmly held by De Kotinsky sealing wax. Fig. 5 shows the thermocouple in place in the animal.

Measurements of the heat level in subcutaneous tissue and skin are relevant to a study of change in body heat, but extreme care must be taken in surface measurements. The proper manner of attachment of the thermocouple to the skin is a matter of concern even with a cooperative subject. With animals the difficulties are multiplied. The wire junction should be appressed against the skin tightly enough to assume the level of skin heat; yet too firm

excised uteri (dog, cat, rabbit, and rat) and found that the benzyl esters only partially relaxed tonus, while the barbiturates caused a complete loss of tonus.

It appears, therefore, that nothing has been reported concerning the effect of paraldehyde on uterine activity, either when used alone or in combination with benzyl alcohol and that no conclusive *in situ* experiments have been reported which deal with benzyl alcohol alone.

#### METHOD OF STUDY

The compounds, benzyl alcohol and paraldehyde, were studied on both isolated and intact uteri; rabbits and guinea pigs were used for the isolated, and rabbits and cats for the intact uterus.

The method adopted for the isolated organ employed two uterine strips, one of which consisted of an entire horn (guinea pig) or a segment of one of the horns (rabbit), the other of a transverse section across the cervical region where the horns unite. These two preparations were then mounted simultaneously in 100 c.c. of a suitable alkaline oxygenated Locke-Ringer solution, kept at a temperature of about 40° C. Each preparation was connected to a light Harvard heart lever for recording movements on a smoked surface. In this manner one lever mechanically described the effects on the longitudinal (horn) and the other on the circular muscles (cervix) under identical conditions. To record the effects on the intact uteri of rabbits and cats, the following procedures were adopted: The animals were first anesthetized lightly with paraldehyde *per os*; rabbits received either 1.7 c.c. of paraldehyde per kilo or this amount of paraldehyde combined with 0.17 c.c. per kilo of benzyl alcohol; cats were given 2 to 2.4 c.c. per kilo of paraldehyde without benzyl alcohol.

When fully anesthetized, the abdomen was opened while immersed in a saline bath maintained at about 40° C.; one uterine horn was exposed under water and connected to a light modified Cushny myocardiograph lever for recording the uterine movements. Both drugs were dissolved in saline when administered intravenously (jugular, cat; marginal ear vein, rabbit) and used pure when given *per os*.

#### RESULTS WITH PARALDEHYDE

*Rabbit (Isolated Uterus).*—Dilute solutions of paraldehyde, 5 to 100 mg., were practically without effect on either the longitudinal or circular preparation, regardless of the functional state of the uterine muscle (pregnant or non-pregnant), although the higher concentration, 100 mg., may cause some increase in both tonus and rate of contraction in the longitudinal segment (Fig. 1).

High concentrations, 200 to 500 mg., usually depress both segments as shown by a decrease in tonus, amplitude and rate of contractions, or a disappearance of the spontaneous contractions with but slight decrease in the tonus of the longitudinal segment (Figs. 2 and 3).

*Guinea Pig (Isolated Uterus).*—Unlike the rabbit, dilute solutions of paraldehyde definitely stimulate, whereas the concentrated solutions stimulate mo-

incident upon handling and placement in the cage, but to movement, is seen when an increase occurs as the animal is permitted to move about within the cage. Handling was not a factor, for the cage opened automatically. Presumably the normal routine movements of the nonimmobilized animal tend to keep the heat level higher than in the resting state. The recordings almost invariably reveal a drop of one to several degrees in the general body temperature after an hour of immobilization.

2 *Avoidance of Excitement*—Excitement gives greater elevation of the heat level than movement, although some amount of motion usually accompanies excitement. When held in the hand of an experimenter, the rat exhibits a general heightening of sympathetic system responses. If, in addition, a temperature testing device is plunged deeply into the rectum, the tempo of heartbeat, heat production, and general activity are further augmented. Invariably, these responses are lessened as the animal becomes tamer and more accustomed to handling, yet, no rat so far tested even when awakened from a period of sleep, has recorded at the onset a temperature as low as the base line level arrived at after an hour of confinement from movement and excitement. Handling and taking of temperature with a thermometer are inherently exciting factors which increase activity and body temperature. They are overcome to a large extent by a technique employing continuous recording of the immobilized, quietly resting animal, the first readings of which are begun after an hour of immobilization.

3 *Continuous Measurement Over Long Periods of Time*—For observation over any extended length of time this technique is extremely valuable since a recording can be printed throughout a long test at any desired period. Readings used in this study have been taken at intervals of fifty three seconds, with one nonprinting interval after every twelfth recording. Thus, quick changes are readily detected in unbroken sequence over long tests without exciting factors incident to each thermometer trial. Continuous measurements have been run on rats for as long as forty eight hours. The thermocouple does not interfere with defecation.

4 *Accuracy and Speed in the Thermal Measurement*—Accuracy may well be increased by replacement of the thermometer with thermoelectric devices which can be built or bought, with a far greater sensitivity than that possessed by the thermometer. Speed in the measurement of small changes in temperature is afforded by the rapid passage of electric currents instead of the pause of minutes before the thermometer reaches an equilibrium.

*Animal Training, Thermocouple Implantation, Pre and Postoperative Care*—That part of the technique involving care of the animals rather than mechanical means has been applied chiefly to the rat, but also to the mouse, guinea pig, rabbit, cat, dog, and monkey.

By means of a thermocouple inserted through the anus colonic readings were obtained at least once every three minutes during tests of internal organs, these serve as a base line level of body heat in the organism. A common base line body temperature, to which all internal parts can be referred, is especially necessary during any change in the level of animal heat, for the internal organs are directly affected by the temperature of the body regions

Additional paraldehyde was given intravenously. Single doses of 2 to 4 c.c. or as much as 10 c.c. of an 8 per cent solution given during the course of one hour were practically without effect (Fig. 7).

These results were uninfluenced by morphine, 15 mg. per animal, given intravenously.

*Cat (Intact Uterus).*—Nonpregnant anesthetized cats were used for these experiments. In this type of animal spontaneous contractions were not always present, or if present were few and weak.

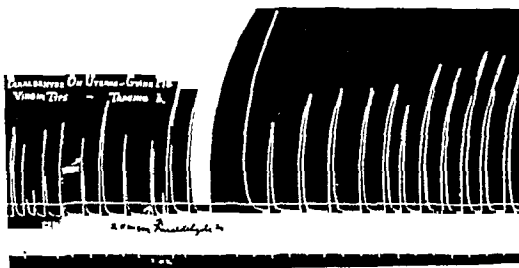


Fig. 4.

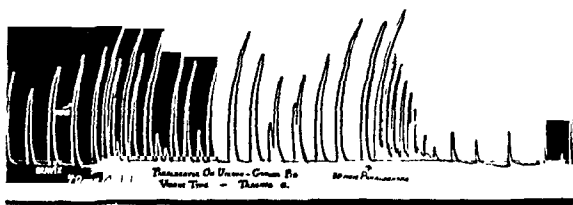


Fig. 5.

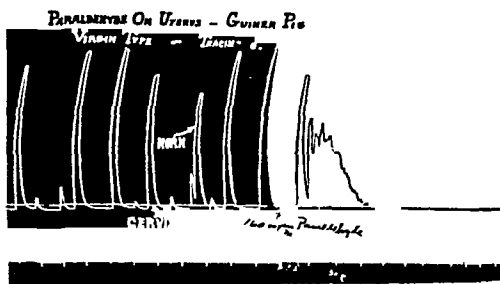


Fig. 6.

Figs. 4, 5, and 6.—Paraldehyde on the isolated uterus of the virgin guinea pig. Mechanical relations same as in the previous figures.

Fig. 4.—Effect of 20 mg.

Fig. 5.—Effect of 40 and 50 mg.

Fig. 6.—Effect of 160 mg. Note that the longitudinal segment (horn) only is stimulated.

In order to produce prominent rhythmic contractions, a small dose of post-pituitary solution was given intravenously.

When contractions were established in this way they were uninfluenced by a single intravenous dose of 6 c.c. of an 8 per cent solution of paraldehyde and only moderately depressed (rate and height of contractions) by repeating this dose within fifteen minutes.

kept the extruding wire from rubbing against the cage walls. Accessory spirals of wire were left just beneath the skin as reserve in case of a break outside of the body (Fig 4)

After thermocouple implantation the rat was kept in a narrow, low roofed cage, which permitted motion backward and forward but prevented assumption of a completely upright position until fibrosis held the wires in place. Organizing processes occurred quickly in the rat, as seen from investigations by Harvey<sup>2</sup> on the return of strength after wound healing.

At the completion of the experiments an autopsy was performed on each animal to check on the retention and condition of the implanted thermocouple.

#### DISCUSSION

The adaptation of the technic to everyday uses is limited. Mention has been made of the errors that may vitiate the accuracy of the methods and of the precautions that must be observed. In addition, there are certain limitations in its practicability. A generous amount of time is required. Animals must be trained for they do not acquiesce at first to immobilization. Some never respond favorably to the training. In order to reach a base line level of body heat, they should lie quietly or be acclimated to placement in an immobilization cage for an hour before tests are begun. Even simple types of recording potentiometers, plus attachments cost approximately \$300.00. Thus it can be readily seen that where records are desired on more than a very limited number of animals, a considerable expenditure of time and money is required as well as a working knowledge of galvanometric and thermocouple circuits. The thermometer remains a much simpler, more facile means if only rectal temperatures are desired.

This method is offered as particularly suitable for rapid readings over long periods, where speed and accuracy in readings are essential. For the measurement of internal temperatures the method is ideal. Application to physiologic problems may be briefly discussed.

Gradients with their direct bearing upon human health and comfort present an urgent and vital series of problems which can be profitable and adequately furthered by combining this technic with the methods now available.<sup>3,4</sup> Investigation of temperatures throughout the subdermal parts of the body should be carried out on a large series of animals of various sizes and habits. Particularly interesting would be readings of gradient and organic warmth in the animals awakening from hibernation with a sudden outburst of revived bodily activities.

Insulated units applying localized heat have been implanted in the interior of the body with thermocouples which register and regulate the warmth in the heated tissue. Preliminary work indicates increased metabolism due possibly to (1) locally increased blood supply and (2) more rapid biochemical reactions at higher temperatures. Localized heating of various nervous centers in the unanesthetized animal, particularly of the hypothalamus, may add to the understanding of heat regulation.

Measurements with this technic may find immediate value in a branch of therapeutics now under intensive investigation, namely, treatment by heat as in

The large doses, 50 to 80 mg., either decreased tonus, rate and amplitude of contractions or caused complete cessation in both circular and longitudinal preparations.

The effects appeared to be the same in both pregnant and nonpregnant animals.

*Guinea Pig (Isolated Uterus).*—The effects of benzyl alcohol on the isolated uterus of the guinea pig were practically like those described for the isolated uterus of the rabbit.

The dosage range was somewhat narrower (4 to 60 mg.).

Fig. 9 illustrates the effects following the application of 60 mg. in the parturient type, the spontaneous movements having stopped in the cervix, but continued in the horn, although feebler; tonus alone may be depressed if the dosage is not excessive.

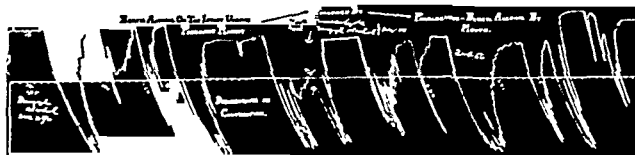


Fig. 11.—Uterine activity after 3 c.c. of a 3 per cent solution of benzyl alcohol given intravenously at 1:37, using the same rabbit as employed for Fig. 7. A second anesthetic dose of the mixture was given by mouth at 2:16. Note absence of effects as late as 2:41. Downstroke, contraction.

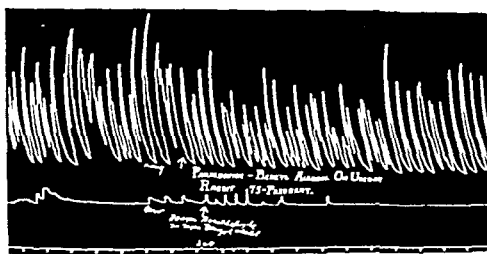


Fig. 12.—Effect of a mixture of paraldehyde (80 mg.) and benzyl alcohol (20 mg.) on the isolated pregnant uterus of the rabbit. Upstroke, contraction. Upper tracing, longitudinal segment; lower tracing, cervical segment.

Fig. 10 illustrates the effects in the virgin type uterus. In guinea pigs the heavy parturient type is less easily depressed than is the thin noncongested virgin type.

*Rabbit (Intact Uterus).*—Animals anesthetized with paraldehyde or with paraldehyde and benzyl alcohol were given as much as 3 c.c. of a 3 per cent solution of benzyl alcohol intravenously without influencing normal spontaneous contractions in either the pregnant or nonpregnant type of uterus (Fig. 11).

*Cat (Intact Uterus).*—A dose of 6 c.c. of 1 per cent benzyl alcohol given intravenously in the nonpregnant cat was without effect on the rhythmic contractions produced by a small dose of postpituitary solution while 6 c.c. of a 3 per cent solution produced moderate depression only (decrease in height and rate of contractions).

## THE EFFECT OF PARALDEHYDE AND BENZYL ALCOHOL ON UTERINE ACTIVITY\*

GEORGE B ROTH, M D AND HOWARD F KANE, M D , WASHINGTON, D C

IT WAS recently reported by Kane and Roth,<sup>1</sup> 1934, that the rectal instillation of a mixture of paraldehyde benzyl alcohol, with or without morphine, may be successfully used for securing obstetric analgesia and amnesia. They further stated that the mixture seemed superior for this purpose to any other existing method.

Laboratory studies made to ascertain the effects of these agents on uterine activity are herein reported.

The mildly depressant activity of paraldehyde on the organism has long been known<sup>2</sup> (Cervello, 1882-1884), the drug ranking in the laboratory as one of the safest hypnotics and fixed anesthetics in common use. However, when used clinically by mouth its disagreeable taste is highly objectionable, particularly to women, and when instilled rectally, the irritant nature of paraldehyde prevents its retention.

Kane and Roth,<sup>1</sup> 1934, found that its expulsion from the rectum could be prevented by adding to it the comparatively nontoxic local anesthetic, benzyl alcohol (about 1 part of benzyl alcohol to 10 parts of paraldehyde). When combined in this way, they obtained retention almost invariably if administered after a cleansing enema.

No references to experimental work on the effect of paraldehyde on uterine activity could be found (either when used alone, or in conjunction with benzyl alcohol), while for benzyl alcohol only two references to its action on the uterus were met with.

Macht,<sup>3</sup> 1918, working with the excised uterus of the rat, obtained a decrease in tone and amplitude from the use of 1 c.c. of a 1 per cent solution of benzyl alcohol in 30 c.c. of Locke's solution. Similar effects were obtained from the use of benzyl benzoate and benzyl acetate which led him to suggest the use of the benzyl compounds for spasmodic contractions of the uterus as in dysmenorrhea or threatened abortion.

Mason and Pieck,<sup>4</sup> 1920, reported that 3 c.c. of a saturated aqueous solution of benzyl alcohol (approximately a 4 per cent solution) given intravenously to dogs had no effect on uterine movements *in situ* but that a 5 c.c. dose "stopped uterine contractions but not until the animal had died." They further state that "we should not be inclined to lay too great emphasis on these observations."

Gruber,<sup>5</sup> 1927, compared the effects of sodium benzyl succinate and sodium dibenzyl phosphate with those of certain commonly employed barbiturates on

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5. Benzyl alcohol given intravenously to rabbits and cats anesthetized with paraldehyde was without effect on the uterine movements in as high a dosage as 3 c.c. of a 3 per cent solution.

6. Simple summation rather than a potentiation of effects results from the use of paraldehyde and benzyl alcohol on the isolated uterine muscle of the rabbit and guinea pig.

7. Both paraldehyde and benzyl alcohol may be considered to be but mildly depressant to uterine muscle, the former being less depressant than the latter.

#### REFERENCES

1. Kane, Howard F., and Roth, George B.: *Trans. Am. Assn. Obst. Gynec. & Abdom. Surg.*, p. 201, 1934.
2. Cervello, V.: (a) *Arch. f. Exper. Path. u. Pharmacol.* 16: 264, 1882-1883. (b) *Arch. ital. de Biol.* 6: 113, 1884.
3. Macht, D. I.: *J. Pharmacol. & Exper. Therap.* 11: 419, 1918.
4. Mason, Edward C., and Pieck, C. E.: *J. LAB. & CLIN. MED.* 6: 62, 1920.
5. Gruber, C. M.: *J. Pharmacol. & Exper. Therap.* 30: 149, 1927.

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### TRICHINOSIS, WITH A REPORT OF TWO CASES WITH EOSINOPHILES IN THE STOOLS\*

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THE finding of eosinophiles in stools has not been frequent in our experience. Some years ago, stool examinations were made for about 1,100 patients in the Newark State School many of which tests were repeated.<sup>1</sup> All of these specimens were examined in the "fresh" state with neutral red and with iodine. During the following years, about 10,000 routine stool examinations were made in the fresh condition and only those which showed cells were permanently stained. In the last three years, we have examined in this laboratory about 250 stools, but these were all from patients who had definite gastrointestinal symptoms, often a severe bloody diarrhea. These also were examined in the fresh condition and, if any unusual cells were found, smears were made, and stained with Wright's or Giemsa's blood stain. In this number of stool examinations, the authors remember only the two cases following as showing eosinophiles.

A laborer, about sixty years old, had had a diarrhea for several weeks. This began in the autumn (1933) soon after he ate a sandwich of raw sausage. He remembers feeling tired and weak, and finding it difficult to do the day's work. He cannot tell anything more of his condition. He continued work and went to see a physician when the diarrhea had continued for several weeks. He finally came to Dr. E. Tansley of Newark who brought a stool specimen to the laboratory. The stool (December 18) was a watery, brown one with some meat fibers, and granular cells which, when stained with

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\*From Newark State School.

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mentarily, then depress uterine motility. The stimulating effect of dilute solutions of paraldehyde is exemplified best in the longitudinal segment of the virgin type uterus.

Figs 4, 5, and 6 illustrate the effects produced by gradually increasing the concentration. When 4 mg were used, the contractions became higher gradually, increasing to 20 mg caused a further increase in height (Fig 4). However, when one uses a dosage range of 80 to 160 mg, depression is seen in tonus, rate and amplitude of contraction preceded by a preliminary increase in rate (Figs 5 and 6).

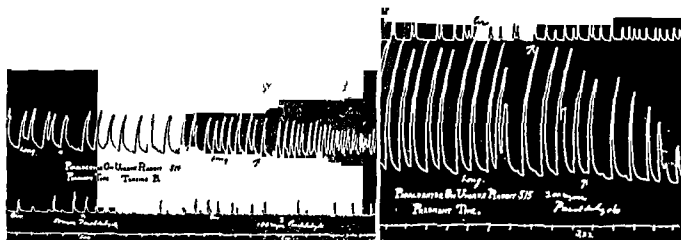


Fig 1

Fig 2

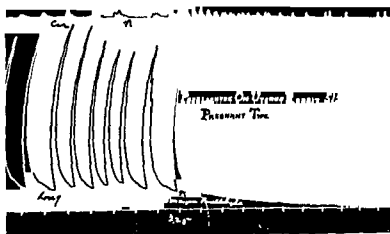


Fig 3

Figs 1, 2 and 3—Paraldehyde on the isolated uterus of the pregnant rabbit. Long longitudinal segment. Cir. circular segment. Upstroke contraction.

Fig 1—Effect of 50 and 100 mg.

Fig 2—Effect of 200 mg.

Fig 3—Effect of 500 mg.

The parturient uterus of the guinea pig appears to be more resistant than the nonparturient type to the depressant effect of paraldehyde, comparatively little depression occurring in the former after as high concentrations as 200 to 300 mg.

*Rabbit (Intact Uterus)*—The uterus of the rabbit (pregnant or nonpregnant) when anesthetized with paraldehyde alone (17 cc per kilo per os) or with this dose of paraldehyde to which was added 0.17 cc per kilo of benzyl alcohol, always showed spontaneous rhythmic contractions as observed when the organ was immersed in a saline bath at 40° C.

eosinophiles. This patient did not have a diarrhea. A blood smear showed 67 per cent eosinophiles. Four days later a very similar count was obtained. June 18, there were 35.7 per cent eosinophiles. The results of blood counts taken at the time of the skin tests are shown in Table I.

Another patient was seen in consultation in June, 1934, with Dr. DuBois (Case 4). She had all the classical symptoms of a trichinosis, fever, malaise, pains in muscles, swollen eyelids, and respiratory difficulty. Seven blood counts taken during her acute illness showed a first count with 6 per cent eosinophiles, gradually increasing to 41 per cent. Her skin test performed in January, 1936, was considered slightly positive. Another case (Case 5, daughter of Case 4) had an eosinophilia of 32 per cent in June (1934). She complained of vague abdominal pains which led to an appendectomy in July, 1935. Her skin test was markedly positive, the largest reaction seen in any of the cases tested (Table II). Another patient (Case 6) of Dr. DuBois was said to have had trichinosis in June, 1934. A blood smear showed 44 per cent eosinophiles. A skin test performed recently gave a strongly positive reaction. A fourth case of Dr. DuBois (Case 7) had trichinosis in September, 1934. She was seen in consultation and considered a typical case. Her eosinophiles, at first 6 per cent, reached 19 per cent three weeks later. The skin test, performed about one and a half years later, was slightly positive.

Nine people with no history of trichinosis infections at any time were skin tested for negative controls. These have all had blood counts recently and no eosinophile increase was found. They all had negative skin tests. Three cases were skin tested because of an eosinophilia. One patient (Case 9) from this institution has had an asthma and a very marked itching of the skin for over two years. Her highest recorded eosinophile percentage (21.0 per cent) occurred the day of the skin test, but she had had 18 per cent some months previously. Another patient (Case 8) had been receiving antisymphilitic treatment and in a routine blood count 23 per cent eosinophiles were found. In the third patient (Case 10) a blood count was done because of a suspected anemia, and 10 per cent eosinophiles were found. These three cases showed positive skin reactions, all reaching a maximum size in five minutes, then fading rapidly; but only in the third case was the reaction as large as was that in those cases who had had or were suspected of having had trichinosis. In none of these three was there a history of an acute or possible trichinous infection.

The antigen for the precipitin test was obtained from O. R. McCoy of the Rochester Medical School, to whom we are greatly obligated. This test was performed according to the method described by Spink and Augustine.<sup>3</sup> A half cubic centimeter of blood serum is overlaid with 0.5 c.c. 1-100 dilution of trichina antigen and incubated one hour at 37° C. A white ring develops in positive cases. As control, 0.5 c.c. of serum was overlaid with salt solution and also incubated.

We had only sufficient antigen to do 10 tests. Six of the cases showed a ring in the antigen tube, none in the control tube (Table II). The three negative controls were laboratory staff members who had negative skin tests, no

## RESULTS WITH BENZYL ALCOHOL

*Rabbit (Isolated Uterus).*—The dosage range employed for benzyl alcohol in this type of experiment extended from 5 to 80 mg. in 100 c.c. of Locke-Ringer's solution. The lower dosages, 5 to 10 mg., had practically no effect;



Fig. 7.—Effect of 4 c.c. of an 8 per cent solution of paraldehyde given intravenously at 12:13 to a pregnant rabbit anesthetized with paraldehyde and benzyl alcohol. Downstroke, contraction. Note that at 12:37 the contractions were still of the sustained type.

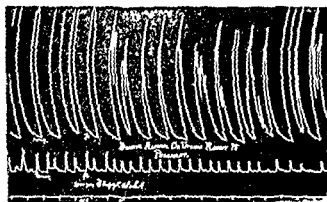


FIG. 8.

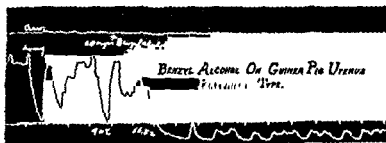


Fig. 9.



Fig. 10.

Figs. 8, 9, and 10.—Benzyl alcohol on the isolated uterus. Mechanical relations same as in Figs. 1 to 6.

Fig. 8.—Effect of 20 mg. on the pregnant uterus of the rabbit.

Fig. 9.—Effect of 60 mg. on the parturient type uterus of the guinea pig.

Fig. 10.—Effect of 20 mg. on the virgin type uterus of the guinea pig.

medium doses, 20 to 30 mg., occasionally caused slight depression and marked depression at other times, the circular being depressed usually before the longitudinal segment (Fig. 8).

history of trichinosis and no record of an eosinophilia. A fourth negative case was one with an eosinophilia, the case receiving antisyphilitic treatment (Case 8).

Discussion: According to these tests, then, the two cases first described had a trichinous infection probably at the time of their diarrhea when the stools were examined and differential leucocyte counts were made.

The interesting point in both cases is the occurrence of diarrhea for weeks and the finding of eosinophiles in the feces. Then it was thought that these might be due to a trichinous infection. At this time, a blood count was requested but smears only were obtained. When a high eosinophilia (45 and 54 per cent) was found, the authors were convinced that these were cases of trichinosis. This opinion was given to the physicians, with the reservation that confirmation was desirable. The confirmatory tests are muscle examination and the more recently described skin and blood precipitin tests with trichina antigen. The finding of trichina cysts in a piece of muscle is the only absolutely positive confirmation. Permission for excision of a piece of muscle could not be obtained. This test, of course, is only diagnostic if encysted embryos are found. At autopsy, such examinations have proved valuable as shown by Queen<sup>4</sup> and others.

The finding of eosinophiles in feces specimens of trichinosis patients is unusual. In a review of various standard textbooks of medicine and laboratory texts, no mention of this was found except in Kolmer and Boerner's recent text<sup>6</sup> who state that eosinophiles may be found in the feces in allergic states. Both patients admitted eating raw or lightly cooked sausage. Neither patient was acutely ill. Further confirmation was obtained by the positive skin reactions and blood serum precipitin tests.

The latter test has added significance to us in that we were able to do them on four patients who had been diagnosed as trichinosis. In these four cases (3, 4, 6, 7), the diagnosis was made by the symptoms, the clinical findings and blood examinations. The patient in Case 5 complained of vague abdominal symptoms at the time of her mother's (Case 4) illness. Several blood counts were taken and all showed an eosinophilic increase, in one count to 32 per cent. We believe this patient also had trichinosis at that time. Her racial origin and family history are confirmatory as were the skin and blood precipitin tests (Table II).

The finding of several<sup>3</sup> positive skin tests in patients with a moderate eosinophilia is confusing. Finding positive skin tests in people with no history has been reported. Negative findings in patients who have had trichinosis have also been recorded. Two of our cases (8 and 9) have been institutionalized for many years, and there have been no known cases of trichinosis in this institution. A cause for their eosinophilia can be given. The third case (10) had no illness, and had what may be considered a high normal eosinophile (10 per cent, 620 eosinophiles) count and a well-marked skin reaction. McCoy and others<sup>5</sup> note that in 3 cases out of 4 with positive skin reactions there was an eosinophilia and comment on this curious finding. They state that allergic patients are not more likely to have positive skin

## RESULTS WITH BENZYL ALCOHOL IN COMBINATION WITH PARALDEHYDE

Benzyl alcohol in combination with or following paraldehyde was tried on both isolated (rabbit and guinea pig, parturient type) and intact uteri (rabbit, pregnant, cat, nonpregnant) the range of dosage in combination on isolated structures being as high as one part of benzyl alcohol in four parts of paraldehyde

The combinations used were 5 to 20 mg of benzyl alcohol to 50 to 80 mg of paraldehyde (Fig 12)

In the intact animals which were anesthetized with paraldehyde, the drugs were given intravenously following each other, or, together by mouth (cat), the oral dose in the cat being the usual anesthetic dose combined with  $\frac{1}{10}$  part of benzyl alcohol The largest single intravenous dose of paraldehyde given to either the rabbit or cat was 6 c c of a 3 per cent solution

The effects on isolated segments when used in the above combinations were practically the same as when used singly, in other words there was no potentiation of the effects resulting from their use in combination

On the intact organ there was no effect in the rabbit or cat (anesthetized) from the intravenous use of as much as 3 c c of a 3 per cent solution of paraldehyde, followed by 3 c c of a 3 per cent solution of benzyl alcohol, or from 43 c c of paraldehyde combined with 043 c c of benzyl alcohol given by mouth to the anesthetized rabbit (Fig 11)

## SUMMARY AND CONCLUSIONS

1 Paraldehyde first stimulates then depresses the isolated uterus of the nonpregnant guinea pig, the stimulant effect being manifested mainly in the horn segment (longitudinal fibers) depression occurring in both the horn and cervical segment (circular fibers) The dosage range for obtaining the stimulant effect was 4 to 80 mg, dosages above 80 mg being depressant

2 Dilute solutions of paraldehyde, 5 to 50 mg, were without effect on either the horn or cervical segments of the isolated uterus of the rabbit (pregnant or nonpregnant), while a 100 mg dose was found at times to be somewhat stimulating to the horn segment Concentrated solutions, 200 to 500 mg, were required to depress motility in either segment

3 Paraldehyde in anesthetic doses when used (a) alone, or (b) in combination with benzyl alcohol (about 10 parts of the former to 1 of the latter), or (c) by supplementing the anesthetic dose by as much as 10 c c of an 8 per cent solution of paraldehyde given intravenously, does not suppress the spontaneous uterine movements of the intact rabbit, while in the anesthetized cat the uterine movements may be present but they appear less seldom than in the rabbit

4 Benzyl alcohol was practically without effect on the isolated uterus of both the rabbit and guinea pig in small dosages, 4 to 10 mg, while depression occurs from the use of larger doses, 20 to 80 mg The qualitative effects appear to be the same in pregnant and nonpregnant animals, but the heavy parturient type uteri were less easily depressed than the thin noncongested virgin type The motility of the cervical segment was usually depressed before that in the horn

ON THE ANTIGENIC RELATIONSHIPS OF HEMOLYTIC  
STREPTOCOCCUS EXOTOXINS FROM DIFFERENT  
PATHOLOGIC CONDITIONS WITH SPECIAL  
REFERENCE TO ERYSIPELAS\*

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THE question of antigenic relationships between toxins of hemolytic streptococci originating from a variety of pathologic conditions has not been settled with finality. Differences of opinion still exist as to the specificity of toxins developed from organisms isolated from cases of scarlet fever, erysipelas, puerperal and other conditions with manifestations of toxemia. The nature of the disagreements is not only theoretical. Its practical importance is connected with the production of therapeutic serums.

It is generally recognized that the stronger toxins produce most potent antitoxins. On the other hand, the general experience has been that the strongest toxins are found among those produced by hemolytic streptococci from scarlet fever. Thus in selecting a strain or strains for the production of antiscarlatinal serums no problem exists as to the source in which such a strain should be looked for.

It has not been so simple with antitoxic serums for other streptococcal diseases, notably for erysipelas. The known hemolytic streptococci from this disease produce only moderately toxic filtrates. The question has arisen whether it is necessary to employ "specific" erysipelas serums produced by strains from erysipelas or whether scarlatinal antitoxic serums possess the same neutralizing capacity for "erysipelatosus" toxins (or for toxins from any hemolytic streptococcal disease) as for scarlatinal.

HISTORICAL NOTES

Numerous studies were made on the question of "specificity" of hemolytic streptococcus toxins. Thus while Birkhaug<sup>1, 2</sup> in 1925 and 1926 and later Dick and Dick<sup>3</sup> in 1929 published their data to show that erysipelas strains represent not only a serologic entity, but that neutralization of toxin by antitoxic serums runs strictly along disease-specific lines, other investigators asserted opposite findings. Ando<sup>4</sup> and Toyoda<sup>5</sup> in 1928 and 1929 did not find any evidence that toxins from hemolytic streptococci from scarlet fever, erysipelas and puerperal fever could be differentiated from each other.

In 1931 Wheeler<sup>6</sup> brought out that a hemolytic streptococcus responsible for many cases of sore throat in an epidemic of this disease was identical with hemolytic streptococcus strains from several cases of scarlet fever that occurred within the same sore throat epidemic. The toxins from both the sore throat and scarlatinal strains were similar to the toxin from a cow's mastitis

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Wright's blood stain, were found to be eosinophiles. Many of these were badly broken up, frequently leaving only a group of red granules. A second specimen four days later was gray watery, and contained many of the clumps of red stained granules and occasionally a well preserved eosinophile.

It was suggested that a blood count be taken but only a blood smear was brought to us. This showed what appeared to be about a normal leucocyte count and, in the differential count there were 54.0 per cent eosinophiles (Case 1, Table I).

For the second case, we are indebted to Dr J. L. Davis of Newark. A white, adult, housewife stated that she had an attack of "flu" in December (1934) which was followed by a diarrhea of several weeks' standing. A stool specimen was brought to the laboratory. This was yellow, liquid, with few meat fibers, a few leucocytes and after staining with Wright's blood stain, clumps of red granules were seen and then eosinophiles found and a blood smear requested. This smear appeared to have a slightly higher than normal leucocyte count and there were 45.0 per cent eosinophiles (Case 2).

The high eosinophile percentage suggested the diagnosis of trichinosis in both of these cases. It seemed desirable to do skin and precipitin tests for trichinosis and to examine a piece of muscle. Following Drake, Hawkes and Warren<sup>2</sup> we obtained antigen from Dr B. Schwartz of the Agriculture Department at Washington who very kindly sent us sufficient dry powder to make antigen for skin tests, and an infected guinea pig.

Skin tests were made on these two cases (December, 1935) and both gave positive reactions with large irregular pseudopods within ten minutes, but which faded in an hour and left no reaction at the end of twenty four hours. As positive controls, we had an equally good reaction on a patient of Dr E. DuBois of Newark who was said to have had trichinosis in June, 1934 (Case 3). Feces examined in this laboratory at that time did not reveal

TABLE I  
SHOWING EOSINOPHILE PERCENTAGES

CASE	DATE	RBC	WBC	NEUTROPHILS	LYMPHO CYTES	MONOS	EOSINO PHILS	BASO PHILS
1 W M	Dec 22 1933			15	29.0	1.0	54.0	0.0
	Dec 3 1935	5.21	7,800	37	48.7	3.3	0.0	0.7
2 D J	Dec 1 1934			21	22.3	0.0	4.0	0.0
	Dec 4 1935	4.83	8,200	60	26.3	1.7	0.0	0.0
3 R W	May 30 1934			28.0	5.0	0.0	67.0	0.0
	Dec 6, 1935	5.70	1,700	56	20.7	0.7	2.0	0.3
4 A F	June 4, 1934	4.30	11,000	26.0	2.0	0.0	41.0	0.0
	Jan 21 1936	4.64	6,900	57	30.7	1.0	1.0	0.0
5 V F	June 16, 1934	4.49	11,700	27	0.5	0.0	22.0	0.0
	Jan 21 1936	4.28	11,000	63	20.0	2.7	1.7	1.0
6 G R	June 7, 1934			29.0	25.0	1.7	44.0	0.0
	Feb 1 1936	4.00	7,000	62.0	4.0	2.7	0.0	0.6
7 M D	Oct 13, 1934			47	28	2.4	19.0	0.0
	Feb 13 1936	4.17	7,200	61.3	37.7	0.7	0.0	0.0
8 N B	Nov 20, 1935	15	9,800	50.0	24	2.7	22.0	0.0
	Dec 2 1935	4.17	8,500	62.3	20	1.0	11.0	0.4
9 M J	July 31, 1935	4.89	9,700	57	19.7	3.0	18.6	1.0
	Dec 2 1935	4.00	9,700	54.0	24	0.3	21.0	0.4
10 E S	May 14, 1935	4.27	6,200	46.3	40.0	3.7	10.0	0.0
	Dec 2 1935	4.44	7,000	47.7	47.7	2.0	6	0.3

it had been ascertained that standardization of these erysipelas filtrates against our own "standard" erysipelas toxin C<sub>3</sub> gave the same results. These toxins were subsequently used for cross-neutralization with different antitoxins, and we found that filtrates with a toxicity below 2,000 S.T.D. in 1 c.c. would not give clear-cut tests. For this reason, we selected 1:200 as a starting point for orientation: All new filtrates were tested in 1:200 and according to the results were considered toxic, weak or negative. If a given toxin in a dilution of 1:200 or higher gave reactions comparable with reactions produced by 1 S.T.D. of the standard scarlatinal toxin, it was considered toxic and the incidence of these was compared with a similar incidence among toxins of scarlatinal origin. If a dilution of 1:200 produced no reactions at all, they were considered "negative" or nontoxic. If, however, a filtrate in a dilution 1:200 gave in susceptible individuals reactions smaller or paler than a reaction produced in the same individual by 1 S.T.D. of the standard scarlatinal toxin, we considered such a filtrate weakly positive. In some instances such weak toxins were retested in dilutions of 1:100 and found in this dilution to produce reactions comparable to those produced by 1 S.T.D. of the standard toxin. These toxins were designated as containing 1,000 S.T.D. per 1 c.c. and placed in the category of "weak" toxins. In order to avoid erroneous results accompanying rough testing of toxins on animal skins, all filtrates were tested on children's skins. Thus of the 72 filtrates tested, we found the distribution in separate groups as shown in Table I.

TABLE I  
72 FILTRATES FROM NONSCARLATINAL HEMOLYTIC STREPTOCOCCI

	ERYSIPELAS		SEPTIC SORE THROAT		OTHERS	
Toxic filtrates (2,000 S.T.D. or more)	36	75.0%	3	75%	5	25%
Weak filtrates (1,000-2,000 S.T.D.)	7	14.5%	0		3	15%
Nontoxic (Less than 1,000 S.T.D.)	5	10.5%	1	25%	12	60%
Total strains in each group	48		4		20	

The significance of these results could be clearly seen from a comparison made with another group of filtrates produced with hemolytic streptococcus strains from scarlet fever. Of 99 strains taken partly from our regular laboratory stock and partly freshly isolated from throats of scarlatinal patients in Willard Parker Hospital, the following distribution was found: 21 toxic (2,000 S.T.D. or more), 17 weak (between 1,000 and 2,000 S.T.D.), and 61 negative. Table II summarizes all results.

At this point we wish to bring out the fallacy of figures based on arbitrarily chosen boundaries for classification as we have selected in this case. As we stated above, 1:200 was chosen by us for separation of the toxic from weakly toxic and nontoxic strains empirically, for this was roughly found to be the lower limit of toxicity suitable for neutralization tests on rabbits. However, an interesting change in proportions appears when we raise the limit to

TABLE II

CASE	DIAGNOSIS	SYMPTOMS	STOOLS	BLOOD	SKIN TEST	PRECIPITIN
1 W M	Diarrhea	Diarrhea	Eosinophiles found	54.0% Eosinophiles	2 years later positive	Positive
2 D J	Diarrhea "Flu"	Diarrhea	Eosinophiles found	45.0% Eosinophiles	1 year later positive	Positive
3 R W	Trichuriasis	Characteristic but no diarrhea	No eosinophiles found	67.0% Eosinophiles	1½ years later positive	Positive
4 A F	Trichuriasis	Characteristic	-	41.0% Eosinophiles	1¼ years later slightly positive	Positive
5 V F	?	Vague abdominal pains Appendectomy July, 1935	-	32.0% Eosinophiles	Strongly positive	Positive
6 G R	Trichuriasis	Characteristic	-	44.0% Eosinophiles	1¼ years later strongly positive	Positive
7 M D	Trichuriasis	Characteristic	-	19.0% Eosinophiles	1 year later slightly positive	Not done
8 N B	Syphilis, being treated	-	-	23.0% Eosinophiles	Positive	Negative
9 M J	Asthma	-	-	21.0% Eosinophiles	Positive	Not done
10 E S	No disease	-	-	10.0% Eosinophiles	Positive	Not done

less large groups of hemolytic streptococci, obviously for their outstanding toxicity, and for this reason we have not included them in the group here presented.

The impression one gains from Table IV is that there is no indication that strains of higher toxicity could not be found among the nonscarlatinal groups if greater numbers were tested. On the other hand, the question of why so many strains isolated from typical cases of scarlet fever (which frequently give almost pure cultures with the first plating) fail to produce toxic filtrates *in vitro*, is not understood by us. If the theory that hemolytic streptococcus exotoxin produces the scarlet fever rash is correct, then the only explanation for the above fact is that none of the artificial media generally in use for the production of toxins *in vitro*, contain that essential element, which exists *in vivo* and which assures the exotoxic manifestations of hemolytic streptococcus in scarlet fever.

2. *Cross-Neutralization of Scarlatinal and Erysipelas Exotoxins With Scarlatinal and Erysipelas Antiserums.*—It has been suggested that cross-neutralization of toxin-antitoxin mixtures from different pathologic conditions is due to inaccurate work with nonstandardized ingredients. In order to clear up this point we planned a series of experiments so as to bring out the element of comparative quantities if any cross-neutralization takes place.

For one of the experiments, we selected cross-neutralization of an erysipelas exotoxin with a known scarlatinal antitoxin and this done alongside with neutralization of a scarlatinal exotoxin with the same antitoxin. The standard toxin 1927 and standard antitoxin B<sub>11</sub> were used as the known reagents, whereas erysipelas exotoxin produced from our erysipelas strain C<sub>3</sub> was selected as the "unknown" toxin. In the very beginning the question of standardization of this toxin came up, so that a proper test dose could be selected. As there was no other practical method available, our first orientation had to be made by comparison with the standard scarlatinal toxin. Thus, we found it to be approximately 5,000 to 7,500 S.T.D. per 1 c.c. With this initial information the test which appears in Protocol 1 was planned and carried out.

These tests were carried out in our early stage of experience with neutralization of toxin on rabbit skins, when each individual rabbit was unknown as to the degree of its skin sensitivity to the toxin. In order to assure definite results, the comparative tests were planned in two series of dilutions, where both the amounts of the toxin test dose (5 S.T.D. and 50 S.T.D.) and the amounts of the neutralizing standard serum were varied. Protocol 1 clearly shows, that whereas the 1:50 dilution of the erysipelas toxin is completely neutralized, with the whole series of the higher standard serum dilutions, similarly to the analogous amount (1:350) of the standard toxin, the larger test dose of the same erysipelas C<sub>3</sub> toxin, the 1:5 dilution is neutralized only with the larger amount of the serum, giving a clear-cut end-point at the same level of the series as in the analogous series with the standard toxin. This holds true in both Rabbits A and B. It will be noticed that there is a slight difference in the behavior of each rabbit skin toward the smaller test doses of

reactions, but they had a large percentage of positive skin reactions on patients with intestinal worm infestation, notably the trichina. Intestinal worms have been excluded by stool examinations as the cause of eosinophilia in these three patients. Such findings of course, throw doubt on the value of skin tests. Further work with skin tests on patients with eosinophilia should be worth while.

Precipitin tests are also indicated as a further check on some of these cases. We have had no false positive results, and our three known trichinosis patients (3, 4, 6) gave positive precipitin reactions. The positive tests in our cases showed a wide diffuse gray band at the junction of the two fluids. One case (Case 8) with eosinophilia and no history of trichinosis gave a negative precipitin test.

#### CONCLUSIONS

Eosinophiles were found in the stools of two cases with weeks' long diarrhea. A history of eating raw sausage just before this diarrhea began was obtained in both, they had high eosinophile percentages in blood smears at that time, and now gave positive skin and precipitin reactions to trichina antigen. Four patients known to have had trichinosis also gave positive skin and three of these positive precipitin tests. One patient (Case 5), an Italian girl, daughter of a known trichinosis case (4), had an eosinophilia at the time of her mother's illness, and now gave a markedly positive skin and precipitin test. Nine patients with no record of an eosinophilia and a history negative for trichinosis all gave negative skin tests and 3 of these had a negative precipitin test. Three patients in whom an eosinophilia (10, 21, and 23 per cent) was found in routine blood counts gave positive skin tests and one of these a negative precipitin test. The finding of positive skin tests in cases with eosinophilia may be accidental, since McCoy and others also noted this but a further study of such cases with skin and precipitin tests is indicated.

#### REFERENCES

- 1 Thomas, W. S., and Baumgartner, E. A. Protozoa in Feces Examinations. *J. A. M. A.* 85: 1725, 1925.
- 2 Drake, E. H., Hawkes, R. S., and Warren, M. An Epidemic of Trichinosis in Maine. *J. A. M. A.* 105: 1340, 1935.
- 3 Spink, W. W., and Augustine, D. L. Trichinosis. *J. A. M. A.* 104: 1801, 1935.
- 4 Queen, F. B. The Prevalence of Human Infection With Trichinella Spiralis. *J. Parasit.* 18: 128, 1931.
- 5 McCoy, O. R., Miller, J. J., and Friedlander, R. D. The Use of an Intradermal Test in the Diagnosis of Trichinosis. *J. Immunol.* 24: 1, 1933.
- 6 Kolmer, J. A., and Boerner, F. Approved Laboratory Technique, New York, 1931, D Appleton Century Co.

the two toxins. Whereas Rabbit A indicates an end-point of 1:200 for the standard toxin and no end-point or complete neutralization for the same point of the  $C_3$  toxin, Rabbit B does just the opposite; there is an end-point for toxin  $C_3$  at 1:200 of the standard serum and no end-point or complete neutralization at the same point for the standard toxin. This reveals only the individual variations of different rabbits in their susceptibility to one or the other toxin. The entire test not only reveals cross-neutralization of an erysipelas toxin with a standard scarlatinal antitoxin, but also brings out quantitative relationships in a comparative test. It also reaffirms the justification of standardizing an erysipelas toxin ( $C_3$ ) against scarlatinal toxin (1927) as was done in this case in the preliminary toxin standardization test on children.

In the next experiment a reversed situation was arranged; one toxin erysipelas  $C_3$  neutralized by an erysipelas antitoxin and cross-neutralized by a scarlatinal antitoxin on the same rabbit at the same time. For a scarlatinal antitoxin a purified concentrated monovalent serum produced with the N.Y.5 was used. This we obtained from the New York State Laboratory. In the absence of a monovalent erysipelas serum, we were compelled to use a purified preparation for a serum, the horse of which received for immunization several erysipelas toxins including the  $C_3$  strain. At no time did this horse obtain a scarlatinal toxin. The results of this experiment are shown in Protocol 2.

In this test similarly to the previous one we again used two different toxin test doses for neutralization: 1:75 (5 S.T.D.) and 1:15 (25 S.T.D.). The margins for dilutions of the two antitoxins were also varied for the two sets:  $\frac{1}{1000}$  to  $\frac{1}{4000}$  and  $\frac{1}{600}$  to  $\frac{1}{1000}$  for the scarlatinal antitoxin and  $\frac{1}{100}$  to  $\frac{1}{400}$  and  $\frac{1}{60}$  to  $\frac{1}{100}$  for the erysipelas antitoxin. This selection of widely different dilutions between the scarlatinal and erysipelas antitoxins was based on previous titrations of these antitoxins which gave about 800 units for the scarlatinal preparation and 60 to 80 units for the erysipelas one. Protocol 2 shows a condition dissimilar to the one observed in Protocol 1: that smaller test doses of the toxins with the higher dilutions of the antitoxin gave more satisfactory results than the larger toxin doses with lower dilutions of antitoxin. (The advantage of employing two sets of toxin + antitoxin mixtures in a test of this nature is thus seen from these two protocols.) The outstanding fact brought out in this experiment is essentially the same as in the previous one: an erysipelas toxin is neutralized by a monovalent antiscarlatinal antitoxin parallelly to its neutralization with an erysipelas antitoxin. This parallelism follows the quantitative relationships of the antitoxins. A scarlatinal antitoxin of approximately tenfold strength requires approximately one-tenth the amount of the weaker erysipelas antitoxin to completely neutralize an erysipelas toxin. To make more certain the unit for unit and test dose relationship, we finally planned the following experiment, for which the reader is referred to Protocol 3.

This experiment consists of the same erysipelas toxin  $C_3$  neutralized by the same erysipelas antitoxin as in test of Protocol 2, but instead of the purified

strain when their neutralization with two antitoxic serums was carried out (one of them being the serum of the diseased cow). The author concluded that there was no evidence for supporting the "disease specificity" of toxins from hemolytic streptococcus from any disease caused by this organism.

In 1932 O'Kell<sup>7</sup> described clinical observations on interrelationships between erysipelas, puerperal fever, and pyogenic infections that made him adopt the "unitarian" point of view and explain the different clinical manifestations of streptococcal toxemias by "subtle conditions of tissue susceptibility."

While the above mentioned studies were concerned in toxins from different diseases, Paik<sup>8</sup> in 1925 and Williams<sup>9, 10</sup> in 1925 and 1929 brought out the structural multiplicity of a given toxin and thus explained the known fact of a toxin completely neutralized by a given antitoxin in some individuals and not neutralized in others. The complex structure of a single toxin was later corroborated by Hooker and Follensby<sup>11</sup> in 1934. The antigenic structure of toxins as it affects their valency was most extensively investigated by Wheeler<sup>12</sup> in 1932. This author and also Wadsworth<sup>13</sup> further studied the effect of different scarlatinal toxins on the valency of antitoxins produced by them. They found that the broad capacity of antitoxins to neutralize toxins from different diseases depended solely on the valency of both irrespective of their pathologic origin.

While Williams in her book *Streptococcus in Health and Disease*<sup>14</sup> especially strongly brings out the endotoxic factor in erysipelas (in addition to the exotoxic), Simmons<sup>15, 16</sup> and Simmons and Lewis<sup>17</sup> publish their observations on the treatment of erysipelas with antitoxic serums. The action of antitoxic serums in conditions characterized by bacterial invasion was studied by Parish and O'Kell<sup>18</sup>. They pointed out that in conditions characterized by bacterial invasion while it would be impractical to attempt treatments with endotoxic (antibacterial) serums due to the great variety of types among different strains, the action of purely antitoxic serums not only effects neutralization of the circulating toxin, but has an arresting effect on the spread of the infection itself. The necessity for "specific serums" particular for each streptococcal disease is denied by them.

#### EXPERIMENTAL

1 *The Incidence of Toxic Filtrates From Scarlatinal and Nonscarlatinal Hemolytic Streptococci*—Seventy two strains of hemolytic streptococci from conditions other than scarlet fever were used for the production of experimental toxins. These included 48 strains from erysipelas, 4 strains from septic sore throat and 20 from cases of septicemia following pneumonia, otitis media, puerperal fever and other pyogenic conditions.

Sterile filtrates were produced following the same procedure which is used for the production of the routine scarlatinal toxins. Standard amounts of tryptic broth (modification of the Douglas and Onslow method) were inoculated with a second broth transplant from blood agar culture, kept in incubator for four days and, after addition of 0.5 per cent phenol, filtered through a Seitz filter. The toxicity of all the filtrates was accurately tested against the scarlatinal standard toxin supplied by the National Institute of Health, after

same series of toxins neutralized by the other serum on another group of rabbits. Later we changed this by reducing the number of toxins in a test but neutralizing them with both serums on the same group of rabbits. We felt that such a procedure would give a better comparison as to the action of the two antitoxins on the same toxins. The results given below are the summary results of these two methods and Protocols 4 and 5 are examples of two such tests.

*C. Dilution of Ingredients.*—As to the amounts of antitoxin and the various toxins employed, the following technic was worked out: All the toxins after preliminary standardization on children were used in two different dilutions, each representing a larger and smaller test dose comparable with similarly larger and smaller test doses of the control toxins N.Y.5 and C<sub>3</sub> used in the test. These larger and smaller toxin test doses varied with different groups of rabbits according to their susceptibility, as described in a previous report.<sup>10</sup> The larger dose was always 10 or 5 times greater than the smaller dose. The use of such two different size doses gives a better insight into the degree of neutralization and brings out finer quantitative distinctions than if only one dilution had been used for rough testing. For the same reason, the neutralizing serum was also used in two different amounts. For practical purposes, we found 1:10 and 1:80 of each serum enough to bring out the facts sought by us. The following two protocols give an outline of a test as done in the earlier and later part of the work. As is seen in the protocols, each test is accompanied by full controls of the test serums neutralizing their own toxins and also each other's toxin (Protocols 4 and 5).

#### PROTOCOL 4

TOXINS	DIRECT TOXIN CONTROLS			TOXINS + ANTISCARLATINAL SERUM N.Y.5 (H.580)				
	TOXIN DILU-TIONS	24 HR.	48 HR.	TOXIN DILU-TIONS	1:10		1:80	
					24 HR.	48 HR.	24 HR.	48 HR.
E 2	1: 5	18 × 20++	15 × 15+>	1: 2.5	10 × 14+	10 × 10+	±	-
	1:25	17 × 20+	14 × 15+>	1:12.5	-	-	-	-
607	1:15	24 × 22++	18 × 20++	1: 7.5	x	-	20 × 22	15 × 15+<
	1:75	15 × 19+<	15 × 15+<	1:37.5	-	-	15 × 17+	-
609	1: 20	20 × 22++	18 × 18+>	1:10	-	-	11 × 15+	15 × 15+<
	1:100	18 × 19+>	15 × 15+>	1:50	-	-	x	x
A (1)	1:10	19 × 20+>	10 × 10+	1: 5	x	-	-	-
	1:50	12 × 12+	13 × 15+	1:25	x	-	-	-
47-N.Y.5	1: 200	20 × 20++	19 × 18++	1:100	-	-	x	-
	1:1000	18 × 19+>	14 × 15+>	1:500	-	-	x	-

The number of toxins tested in each rabbit varied with the size of the rabbit; usually two or three unknown toxins were tested. With larger animals we were able to include four toxins besides the N.Y.5 and C<sub>3</sub> controls. As a result of these tests the following figures were obtained:

- |  |    |
|--|----|
| 1. Total number of nonscarlatinal toxins   | 72 |
| 2. Total number of standardized toxins cross-tested with scarlatinal and erysipelas serums | 54 |

TABLE II

	99 TOXINS FROM SCARLET FEVER STRAINS		72 TOXINS FROM CONDITIONS OTHER THAN SCARLET FEVER	
	NO OF CASES	PER CENT	NO OF CASES	PER CENT
2,000 STD or more	21	21.6	44	61
1,000-2,000 STD	17	17.0	10	14
Less than 1,000 STD	61	61.6	18	25
Total	99 strains		72 strains	

a higher level of toxicity, for instance if instead of 1,200 we should select 1,300 as a dividing point between toxic and weak filtrates as Table III shows

TABLE III

	99 TOXINS FROM SCARLET FEVER STRAINS		72 TOXINS FROM ERISIPELAS AND OTHER CONDITIONS	
	NO OF CASES	PER CENT	NO OF CASES	PER CENT
3,000 STD or more	16	16.1	13	18
Between 1,000 and 3,000 STD	22	22.25	41	57

The percentage of "toxic" strains in the nonscarlatinal group drops from 61 per cent to only 18 per cent which is about the same as in the scarlatinal group. On further analysis of these "toxic" strains it is found (Table IV) that while there is slight numerical prevalence in the lower and middle brackets in the nonscarlatinal group higher toxins containing 10,000 to 15,000 STD are found only in the scarlatinal group.

TABLE IV

	16 TOXIC SCARLATINAL STRAINS		13 NONSCARLATINAL STRAINS	
	NO OF CASES	PER CENT	NO OF CASES	PER CENT
3,000-5,000 STD	7	7.5	7	9.8
5,000-10,000 STD	5	5.3	6	8.3
10,000-15,000 STD	4	4.3	0	0

We are bringing out these points because they are in contrast with the prevailing idea: first that scarlatinal filtrates are in general more frequently toxic than nonscarlatinal, and second that individual scarlatinal filtrates are considerably higher in toxicity than the nonscarlatinal. As Table III shows, the first argument is not justified in our experience with 99 scarlatinal and 72 nonscarlatinal strains. As to the second argument, the same table does show that filtrates with a toxicity above 10,000 STD are not found in this group of 72 nonscarlatinal strains, whereas 4 per cent of the 99 scarlatinal strains produce toxins within the limits of 10,000 and 15,000. Neither the superiority in potency nor the incidence of these "superior" toxins is outstanding enough to be used as a differentiating characteristic. However, it should be mentioned that the three strains of *Streptococcus hemolyticus* known to produce the most powerful toxins (not included in the above tables) also belong to the scarlatinal group, the Dochez NY 5 strain, the Dick I strain, and the so-called 2<sub>(2)</sub> strain (isolated by Dr. Anna Williams from an early case of scarlet fever). These strains were already selected by their discoverers from other more or



## Protocol 1\*

RABBIT	TIME OF READING	TOXIN ALONE		STANDARD SCARLATINAL TOXIN 1927		TOXIN ALONE		ERYTHROLAS TOXIN C <sub>2</sub>					
		1 700	0	1 350 (5 STD)	1 35 (50 STD)	1 700	0	+ STANDARD SERUM B <sub>11</sub>					
								1 50	1 100	1 200	1 25	1 50	1 5
A	24 hr	15 × 20	-	-	±	±	±	1 50	1 100	1 200	1 25	1 50	1 75
	48 hr	20 × 26	-	-	±	±	±	-	-	×	-	±	±
	24 hr	25 × 20	-	-	±	±	±	-	-	-	-	±	15 × 15
	48 hr	17 × 23	-	-	±	±	±	-	-	10 × 10	-	10 × 10	20 × 20
B	24 hr	25 × 20	-	-	±	±	±	-	-	8 × 10	-	13 × 15	25 × 25
	48 hr	17 × 23	-	-	±	±	±	-	-	8 × 10	±	13 × 15	25 × 25

\*The negative serum controls are omitted from the protocol

## Protocol 2

RABBIT	TIME OF READING	TOXIN ALONE		STANDARD SCARLATINAL TOXIN 1927		TOXIN ALONE		ERYTHROLAS TOXIN C <sub>2</sub>					
		1 700	0	1 350 (5 STD)	1 35 (50 STD)	1 700	0	+ ANTISCARLATINAL TOXIN (1790)					
								1 50	1 100	1 200	1 25	1 50	1 5
C	24 hr	15 × 20	-	-	±	±	±	1 50	1 100	1 200	1 25	1 50	1 75
	48 hr	17 × 23	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
	24 hr	25 × 20	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
	48 hr	17 × 23	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse

## Protocol

RABBIT	TIME OF READING	TOXIN ALONE		STANDARD SCARLATINAL TOXIN 1927		TOXIN ALONE		ERYTHROLAS TOXIN C <sub>2</sub>					
		1 700	0	1 350 (5 STD)	1 35 (50 STD)	1 700	0	+ ANTISCARLATINAL TOXIN (1790)					
								1 50	1 100	1 200	1 25	1 50	1 5
D	24 hr	15 × 20	-	-	±	±	±	1 50	1 100	1 200	1 25	1 50	1 75
	48 hr	20 × 26	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
	24 hr	25 × 20	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
	48 hr	17 × 23	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
E	24 hr	25 × 20	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse
	48 hr	17 × 23	-	-	±	±	±	-	-	10 × 15	-	15 × 15	diffuse

## SUMMARY

1. In a group of 99 scarlatinal and 72 nonscarlatinal (predominantly erysipelas) hemolytic streptococcus filtrates a proportionally greater number of "nontoxic" filtrates (containing less than 1,000 S.T.D.) was found in the scarlatinal group.

2. Toxins with moderate toxicity (between 2,000 S.T.D. and 3,000 S.T.D.) are more frequent in the erysipelas group, while the 10,000 to 15,000 S.T.D. per 1 c.c. toxins are found in 4 per cent of the entire scarlatinal group and in none of the nonscarlatinal group.

3. Scarlatinal exotoxins 2<sub>(2)</sub>, N.Y.5, Dick II and erysipelas exotoxin C<sub>3</sub>, cross-neutralized with different antisera produced with N.Y.5, and C<sub>3</sub> strains, show antigenic interrelationships qualitatively and quantitatively similar.

4. Eighty-seven per cent of a group of 37 nonscarlatinal toxins including 85 per cent of 31 erysipelas toxins are equally affected by either a scarlatinal (N.Y.5) or an erysipelas (C<sub>3</sub>) antiserum.

5. An erysipelas antitoxin (37F) does not neutralize some of the erysipelas hemolytic streptococci filtrates, which are neutralized by an antiscarlatinal serum.

## CONCLUSIONS

1. Facts are presented confirming the findings of other workers that the exotoxins of hemolytic streptococci, although differing qualitatively, are not disease specific.

2. Hemolytic streptococci from any source are rarely strong toxin producers under laboratory conditions; the few known strong toxin producing strains belong to the scarlatinal group.

3. Since the most potent antitoxic sera are produced by strong toxins, the presented facts point to the selection of strongest known scarlatinal hemolytic streptococcus toxin producers for manufacturing of antitoxic sera against erysipelas or any other disease with symptoms of hemolytic streptococcus toxemias.

4. For the neutralization of the erysipelas exotoxins not neutralized by either erysipelas C<sub>3</sub> or scarlatinal N.Y.5 antisera, the finding of strong toxin producers of correspondingly different antigenicity is necessary. Such strains irrespective of their pathologic source may also serve to neutralize the scarlatinal exotoxins not covered by N.Y.5. Researches in this direction are in progress.

## REFERENCES

1. Birkhaug, R. E.: *Biology of Streptococcus Erysipelas*, Bull. Johns Hopkins Hosp. 36: 248, 1925; 37: 85 and 307, 1925.
2. Birkhaug, R. E.: *Erysipelas, Etiology and Treatment With Antistreptococci Serum*, J. A. M. A. 86: 1411, 1926.
3. Dick, G. F., and Dick, G. H.: *Specificity of Scarlet Fever Toxin Produced by Hemolytic Streptococci*, J. A. M. A. 93: 1784, 1929.
4. Ando, K.: *Relationships of Toxins of Different Hemolytic Streptococci as Brought Out by Test on Human Subjects*, J. Immunol. 15: 191 and 217, 1928.
5. Toyoda, T.: *Experimental Researches on Etiology of Scarlet Fever*, 1929, Isolation Hospital of Kwantung Govt. Dairen, Manchuria, p. 83.
6. Wheeler, M. W.: *Streptococci Associated With Epidemic Septic Sore Throat, Their Relationship to Streptococcus Associated With Scarlet Fever*, J. Prev. Med. 5: 181, 1931.
7. O'Kell, C. C.: *Hemolytic Streptococci in Infective Disease*, Lancet, pp. 761, 815, 867, 1932.

and concentrated antiscarlatinal preparation, the standard antitoxin B<sub>11</sub> was used. This test of one toxin being neutralized by two different antitoxins is supplemented by another toxin Stand 1927 neutralized by the same Stand B<sub>11</sub> antitoxin as a check on the whole system. The results are clear. In the first two columns of Rabbit D a slight excess of neutralization by the erysipelas serum over the scarlatinal serum is apparent. In Rabbit E there is even more correspondence between the amounts of different serums neutralizing the erysipelas toxin, while the last column in the protocol suggests a tendency in the standard scarlatinal serum of better cross neutralization of the erysipelas toxin than of direct neutralization of its own toxin.

3 *The Incidence of Hemolytic Streptococcus Exotoxins Similarly Neutralized by Scarlatinal and Erysipelas Antiserums*—The tests included in the above protocols were selected from a number of similar tests bringing out the same point. These serums had either NY 5 or C<sub>3</sub> as their antigen and gave similar neutralization of the test toxins whether scarlatinal NY 5 2<sub>(2)</sub> and Dick II or erysipelas C<sub>3</sub>. This similarity was determined on the basis of unit value and skin test doses instead of simply "dilution" of an ingredient irrespective of its potency. Next we were interested to see whether the overlapping relationships described above were confined to the small group of exotoxins previously studied or whether they extended to most of the known toxins. We therefore decided to cross neutralize all the available nonscarlatinal (erysipelas and others) toxins with the two representative antitoxins, NY 5 and C<sub>3</sub>, and compare the numbers of positively neutralized toxins on each side.

#### TECHNIC OF NEUTRALIZATION TESTS

A *General Considerations*—In the early part of this study cross neutralization tests on rabbits were done with the precision of quantitative tests described above. However, we felt that for practical purposes this was not necessary. Even with a much reduced number of dilutions for each individual toxin a number of rabbits would have to be used for only two or three toxins under investigation. Often a test had to be repeated because the particular toxin test dose used for neutralization and indicated in a preliminary ear test for the rabbit's susceptibility, was not justified in the test proper, by being too small or too large. On the other hand, we would come across rabbits that were not susceptible to the particular toxin while showing clear cut susceptibility for the standard toxins, NY 5 or C<sub>3</sub>. In such cases the same test would be repeated several times until satisfactory, clear cut results would appear. Occasionally a filtrate that was clearly shown to be in the 2,000 to 5,000 STD grade of toxins, according to tests on human beings, was not toxic for rabbit skins at all or gave only uncertain results. We therefore classified all the neutralized and cross neutralized filtrates as positive, negative, partial, and uncertain, meaning that they were respectively neutralized, not neutralized, only partially neutralized giving a reduced reaction when mixed with the particular serum, and finally the repeatedly nonclear cut results were classified as uncertain.

B *Arrangement of Test*—In the beginning of this study a series of toxins was neutralized by only one of the two serums on a group of rabbits and the

daily injections of 1 c.c. of liver extract or milk protein for seven days, while the controls were given 1 c.c. saline daily for the same length of time. Counts were then repeated on the entire group, and they were killed and bone marrow smears were made from the femur. For the experiment with benzol and the substance to be tested, another group of rats was selected, and all were given daily injections of 1/6 c.c. of benzol in olive oil until the total leucocyte count in the blood was about 400. This required about 7 injections. Several of these rats were killed and the bone marrow was found to be almost devoid of polymorphonuclear leucocytes. One-half of the remaining group were now given daily injections of 1 c.c. of liver extract or milk protein for seven days and the other half were given 1 c.c. of saline for the same length of time. Counts were then done and bone marrow smears made as above. In doing the differential count, Jenner Giemsa stain was employed and the polymorphonuclear leucocytes were classified according to the Schilling index. The bone marrow smears were made on slides and stained with Jenner Giemsa stain, the Giemsa stain being left on the slide for one-half hour. A differential count of 1,000 cells was done, using a square diaphragm in the ocular, such as is used in enumerating reticulocytes.

The experimental data are shown in Table I. Ten animals were used in each group.

TABLE I

		AVERAGE W.B.C. BEFORE INJECTION	AVERAGE W.B.C. AFTER INJECTION	AVERAGE NEUT. POLY. PER CENT BEFORE INJECTION	AVERAGE NEUT. POLY. PER CENT AFTER INJECTION	AVERAGE NEUT. POLY. PER CENT IN BONE MAR- ROW AT AUTOPSY
Exper. 1	Rats injected with milk protein	2,800	4,200	15.5	25.7	32.8
Exper. 1	Control rats in- jected with saline	4,800	6,000	12.7	24.4	27.3
Exper. 2	Rats injected with liver ex- tract	10,000	14,000	32.6	35.0	31.1
Exper. 2	Control rats in- jected with saline	10,100	14,500	33.7	34.9	32.6
Exper. 3	Rats injected with benzol followed by milk protein		5,800		54.3	26.8
Exper. 3	Control rats in- jected with benzol fol- lowed by sa- line		6,000		33.2	27.7
Exper. 4	Rats injected with benzol followed by liver extract		12,600		55.4	34.8
Exper. 4	Control rats in- jected with benzol fol- lowed by sa- line		8,100		33.5	29.3

Of the 54 cross tested toxins

- 3 Total number cross neutralized toxins with definite results 27  
4 Total number of uncertain results 17

In the 37 cross neutralized toxins with definite results, the following relationships were found (Table V)

TABLE V  
RESULT OF CROSS NEUTRALIZATION OF 37 NONSCARLATINAL TOXINS

	WITH SERUM N 1 5	WITH SERUM C <sub>3</sub>	NUMBER IN EACH GROUP	PER CENT IN EACH GROUP	TOTAL PER CENT
	Toxins Similarly Neutralized				86
Neutralized with either serum	+	+	19	50	
Neutralized with neither serum			7	20	
Partially neutralized with either	±	±	6	16	
	Differently Neutralized Toxins				14
Neutralized with N 1 5 only	+	or +	3	8	
Neutralized with C <sub>3</sub> only	or +	+	2	6	

Table V shows that of a total of 37 nonscarlatinal heterogeneous toxins 32 or 86.5 per cent act similarly as to their neutralization with either the erysipelas or scarlatinal antiserum. If we separate from this heterogeneous group 31 erysipelas toxins, we find 26 or 85 per cent similarly affected by either the scarlatinal or the erysipelas antiserum.

Before summarizing and drawing conclusions we wish to present one more protocol which will emphasize the following point. While erysipelas exotoxins are neutralized by a scarlatinal antiserum produced with a suitable strain of hemolytic streptococcus (in this case NY 5), not all erysipelas exotoxins are neutralized by any erysipelas antitoxin produced with any erysipelas strain. The following is an illustration. A horse immunized with two erysipelas toxins, C<sub>3</sub> and 37F, has developed considerable antibodies against 37F and almost none against C<sub>3</sub>. This serum was used by us in a test for the action of the 37F factor on other erysipelas exotoxins. Protocol 6 represents one of a limited number of tests so far tried with erysipelas and other toxins.

The three toxins included in this protocol are all of erysipelas origin. Toxin C<sub>13</sub> while completely neutralized with the scarlatinal NY 5 antiserum, is only slightly affected by serum 37F. Toxin C<sub>27</sub>, also neutralized by the scarlatinal serum, is not neutralized by the erysipelas serum, and finally toxin C<sub>63</sub> is almost completely neutralized by both. There are other indications that strain 37F (erysipelas) producing an exotoxin with factors different from our erysipelas C<sub>3</sub> exotoxin is similar to some scarlatinal exotoxins which in turn are different from NY 5 or Dick II scarlatinal toxins. The study of these "different" toxins, delayed for the lack of antisera, will be reported elsewhere.

# LABORATORY METHODS

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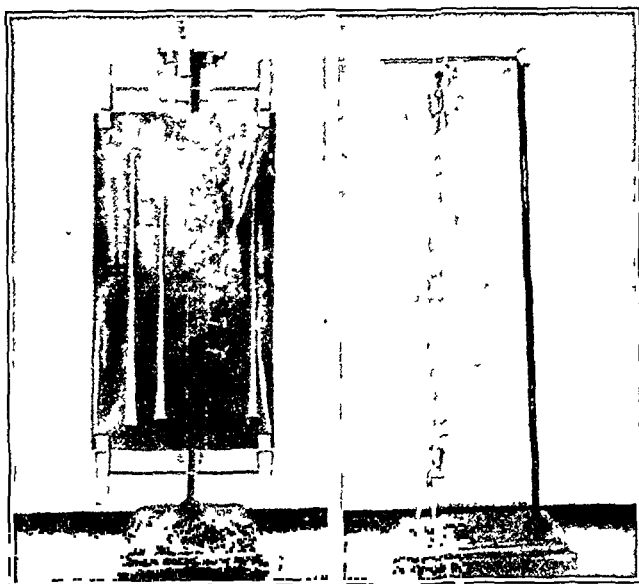
## PREPARING PERMANENT SMOKED-PAPER TRACINGS\*

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VINES COLLIER, JR., WASHINGTON, D. C.

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THE satisfactory preparation of permanent smoked-paper tracings, such as myographs or kymographs, is extremely difficult. Particularly is this true in teaching laboratories of physiology and pharmacology where a hundred or so such records are made each period. After careful study and experimentation, I have evolved a system which is now being regularly employed in the physiology laboratory here at Georgetown with perfect success.



We normally have about 120 students working in the main laboratory, so we have set aside a small room, just off from this laboratory, to be used for preparing records. Here the glazed paper, which has been affixed to the kymograph drums, is smoked over a benzene flame in the usual manner. After the student has completed his tracing he cuts the paper from the drum and fastens a clip on each end.

These clips are made from ordinary spring clothespins in the following manner. A piece of brass rod or tubing of  $\frac{1}{8}$  inch diameter is cut  $\frac{1}{2}$  inch shorter than the width of the glazed paper used. We use paper  $7\frac{1}{4}$  inches wide so the rods are cut in  $6\frac{3}{4}$  inch pieces. A spring clothespin is soldered

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## PROTOCOL 6

TOXINS	DIRECT TOXIN CONTROLS					TOXINS + ANTISCARLATINAL SERUM N x 5 (H 580)					TOXINS + ANTILYSIDELAS SERUM 37F (H 4915)				
	TOXIN DILUTIONS		24 HR	48 HR	TOXIN D	1 10					1 10				
	1:5	1:25	1:5	1:25		24 HR	48 HR	24 HR	48 HR	24 HR	24 HR	48 HR	24 HR	48 HR	24 HR
C <sub>11</sub>	1:5	20 x 22++	18 x 20++	18 x 20++	-	-	-	x	7 x 9	15 x 15+	13 x 14+	13 x 14+	10 x 12+	13 x 15+	48 HR
	1:25	14 x 12++	15 x 10++	15 x 10++	-	x	-	x	-	11 x 12+	13 x 14+	13 x 14+	x	5 x 9	
C <sub>21</sub>	1:5	20 x 22++	13 x 13+	13 x 13+	-	x	x	-	-	12 x 13+	x or +	13 x 16++	15 x 18++		
	1:25	15 x 16++	12 x 15+	12 x 15+	-	x	-	-	-	10 x 14+	x	12 x 18+	9		
C <sub>31</sub>	1:5	17 x 19++	15 x 20++	15 x 20++	-	8 x 10	x	10 x 10+	x	x	x	-	x	+	
	1:25	12 x 15++	12 x 12+	12 x 12+	-	-	-	x	-	-	-	-	-	-	
Control	1:150	15 x 15	18 x 20	18 x 20	-	-	-	-	-	-	-	-	-	-	
47 N.Y.5	1:750	-	-	-	-	-	-	-	-	-	-	-	-	-	
Control	1:5	16 x 20++	16 x 17++	16 x 17++	-	-	-	-	-	10 x 10+	x	x	9 x 10+	x	
37 F	1:25	15 x 16++	15 x 19++	15 x 19++	-	-	-	-	-	9 x 10+	x	x	x	x	

# STUDY IN THE DIFFICULTIES ENCOUNTERED IN THE FRIEDMAN TEST, AND A NEW MODIFICATION USING BLOOD SERUM\*

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SINCE the original Aschheim-Zondek test, there has been one modification worthy of note, namely, the Friedman test. It has been reported as being practically infallible after the tenth day, following the first missed period following suspected conception.

In this test the rabbit is killed and autopsied twenty-four to thirty-six hours after injection, preferably thirty-six hours. Since in some instances a positive result is obtained in twelve hours, it has been recommended to inject two rabbits; the first, autopsied at twelve hours; the second, at twenty-four or thirty-six hours. In some communities laboratories have found it unprofitable to breed and raise rabbits, therefore purchasing them from some local dealer. Even then, the cost has been formidable, so that only one animal has been used, it being held and killed at the maximum time limit of thirty-six hours.

Many workers have encountered difficulties other than that of obtaining suitable rabbits, the principal ones being: (1) Shock following injection of the urine, and (2) false negative or positive reactions. Hoffmann described a technic which eliminates most of these difficulties, particularly shocks, and false negative reactions.

## SHOCK FOLLOWING INJECTION OF URINE

There may be four possible contributing causes of death to the rabbit due to injection of urine.

1. *Rapid Injection of the Urine.*—It is advisable to give the injection extremely slowly; better, to inject 2 or 3 c.c. of urine, followed six or twelve hours later with an additional injection of the same quantity.

2. *Large Doses of Aspirin; or, Quinine Taken by the Patient to Produce Menstruation.*—This has a tendency to kill test rabbits. Probably the only way to overcome this is to instruct the patient to abstain from either of these drugs at least twelve hours before procuring the specimen.

3. *Inbred Rabbits.*—Only animals of the strongest constitution and raised under the most healthful conditions should be used. Inbred rabbits of a bad strain will succumb to injections of urine regardless of the caution used in slow injection. For this reason, it is advisable to procure a new stock of male rabbits for breeding once a year.

4. *Reaction of the Urine.*—The reaction of urine should be adjusted by the addition of N/10 HCl, or N/10 NaOH until the pH lies between 6.8 and 7.4. Extremely acid or alkaline urines will tend to kill test rabbits.

\*Received for publication, April 23, 1936.

- 8 Park, W H, and Spiegel, R G Complexity of Scarlet Fever Toxin & Antitoxin, *J Immunol* 10 829 1925
- 9 Williams, Anna W Relationship Between Different Antibodies, *J Public Health* 15 129, 1925
- 10 Williams, Anna W Exotoxin of Hemolytic Streptococci, *J A M A* 93 1544, 1929
- 11 Hooker, S F, and Follensby, E M *J Immunol* 27 177, 1934
- 12 Wheeler, M W Antigenic Activity of Hemolytic Streptococci From Different Types of Infections, *J Immunol* 24 311 1932
- 13 Wadsworth, Augustus B, Kirkbride Mary B and Hendry, Jessie L A Comparative Study of the Potency and Polyvalency of Antistreptococcus Serum, *Am J Hyg* 9 371, 1929
- 14 Williams, Anna W Streptococcus in Health and Disease, 1933, Williams and Wilkins
- 15 Symmers, D The Antitoxin Treatment of Erysipelas *J A M A* 91 535, 1928
- 16 Symmers, D, and Lewis, K M Antitoxin Treatment of Erysipelas, *J A M A* 99 1082, 1932
- 17 Symmers, D, and Lewis, K M The Antitoxin Treatment of Erysipelas With 4698 Observations, *M Clin North America* p 861 1934
- 18 Parish, H J, and O'Kell, C C Two Studies of Streptococcal Infections, *Lancet*, p 746, 1928
- 19 Kolchin, B S Use of Rabbits in Standardization of Antiserum of Hemolytic Streptococcus, *J Immunol* 24 397 1933

## FURTHER STUDIES IN THE TREATMENT OF AGRANULOCYTOSIS\*

### THE EFFECT OF INJECTIONS OF LIVER EXTRACT AND MILK PROTEIN ON THE BLOOD AND BONE MARROW OF THE RAT

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**I**N A previous paper<sup>1</sup> the authors reported on the stimulating and maturative effect of injections of pentnucleotide on the hematopoietic system of the rat. The practically negative experimental results cast some doubt on the value of pentnucleotide in the treatment of agranulocytosis.

At present, injections of liver extract and milk protein are being widely used to stimulate the production and improve the maturation of granulocytes. Our clinical experience with these substances has not been very conclusive, and we decided to try their effects on the blood and bone marrow of the rat.

On one group of animals the effects of repeated injections of large doses of liver extract or milk protein alone were studied, while in another group the bone marrow was first depressed with benzol before the substance was injected. Control groups of rats in which saline was injected were run at the same time for each group. The peripheral blood and bone marrow were examined in each case for evidence of stimulating or maturative effects of the injections on the blood cells. In the blood the Schilling count was employed as a criterion of these changes, while the bone marrow cells were studied in smears.

*Technic*—The rats used were about eight to ten weeks old. For the experiment with liver extract or milk protein alone, single counts were done on all the animals before the injections were begun. One half were then given

\*From the Crocker Institute of Cancer Research, Columbia University, and the Medical Services of Lenox Hill Hospital and City Hospital.

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# THE ACCURACY OF A NEW TECHNIC FOR MEASUREMENT OF RED BLOOD CORPUSCLE SEDIMENTATION\*

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THIS investigation was undertaken to ascertain the limits of accuracy, and the factors influencing the determination of the sedimentation rate of red blood corpuscles, as measured with a new micropipette previously devised by one of us.<sup>1</sup>

For this study both human pneumonia blood and dog blood were used. Potassium oxalate (1 drop of a 20 per cent solution per 1 c.c. of blood) or heparin† (1 drop containing 6 cat units per 1 c.c. of blood) was used as the anticoagulant. Each experimental result represents the mean of three simultaneous determinations. The results are graphically shown by continuous curves which are plotted to show changes in percentage of sedimentation of red blood corpuscles per unit time.

Fig. 1 represents a typical sedimentation curve of oxalated blood from a pneumonia patient. This curve illustrates three distinct phases of the blood sedimentation: first (*A*) acceleration or induction; second (*B*) the phase of maximal speed of settling; and third (*C*) the phase of slowing and cessation.

Table I illustrates the results of six simultaneous determinations to show the limits of accuracy of the sedimentation curves. The greatest variation is observed during the period when the red blood corpuscles are falling at their

TABLE I  
REPRODUCIBILITY OF SEDIMENTATION READINGS

TEMPERATURE	MINUTES	MEAN	DEVIATION FROM MEAN	
26° C.	0	0	0	
	3	2.1	-1.1	+1.9
	4	2.7	-0.7	+2.3
	6	5.0	-2.0	+2.0
26° C.	7	6.7	-2.7	+3.3
	9	10.7	-3.7	+4.3
26° C.	11	15.6	-4.6	+5.4
	13	20.3	-3.3	+4.7
	15	25.5	-3.5	+4.5
	17	20.1	-3.1	+4.9
26.5° C.	19	34.8	-2.8	+4.2
	22	41.0	-2.0	+3.0
26.5° C.	25	46.7	-1.7	+1.3
	30	52.0	-2.0	+2.0
	35	55.1	-1.1	+0.9
	45	58.7	-1.7	+2.3
26.0° C.	60	60.7	-1.7	+2.3
26.0° C.	90	62.7	-1.7	+2.3
	105	63.3	-1.3	+2.7
25.5° C.	120	63.5	-1.5	+2.5

\*From the Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center.

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†The heparin employed contained 15 cat units per milligram.

## DISCUSSION

A study of the experimental data reveals that in the rat injections of milk protein have very little effect in stimulating or improving the maturation of the white blood cells. The total white count, Schilling index and bone marrow differential react about the same after saline injections as after milk protein. Regeneration after benzol poisoning occurs almost as quickly with saline injections as with milk protein.

Liver extract, first used with apparent success by Foran, Sheaff and Trimmer,<sup>2</sup> gives a better response. Its effect is especially marked after benzol poisoning. The liver extract probably acts by stimulating the bone marrow, since the number of polymorphonuclears is increased, not only in the peripheral blood, but in the bone marrow as well. However, the results with liver extract are far from startling, and it is not surprising that Jackson and Parker<sup>3</sup> and Heck<sup>4</sup> found it to be of little value in their cases of agranulocytosis. Nevertheless, there are no detrimental effects following intramuscular injections of this substance, so that cases of agranulocytosis should be given the benefit of a trial with such therapy.

## CONCLUSIONS

1. Injections of milk protein were of very little value in stimulating or improving the maturation of the granulocytes of the rat.

2. Injections of liver extract had a moderate stimulating effect on the blood and bone marrow of the rat.

3. Cases of agranulocytosis should be given the benefit of a trial with liver extract injection therapy.

## REFERENCES

1. Reich, C., and Reich, E. The Hematopoietic Response of the Rat to Injections of Pentnucleotide, and Its Relation to the Treatment of Agranulocytosis, *Am J M Sc* 71: 188, 1934.
2. Foran, F. L., Sheaff, A. M., and Trimmer, R. W. Agranulocytic Angina: Treatment by the Use of Parenteral and Oral Liver Extract. *J A M A* 100: 1917, 1933.
3. Jackson, H., and Parker, F. Agranulocytosis: Its Etiology and Treatment, *New England J M* 212: 137, 1935.
4. Heck, F. J. Recurrent Granulocytopenia, *Proc Staff Meet, Mayo Clinic* 9: 200, 1934.

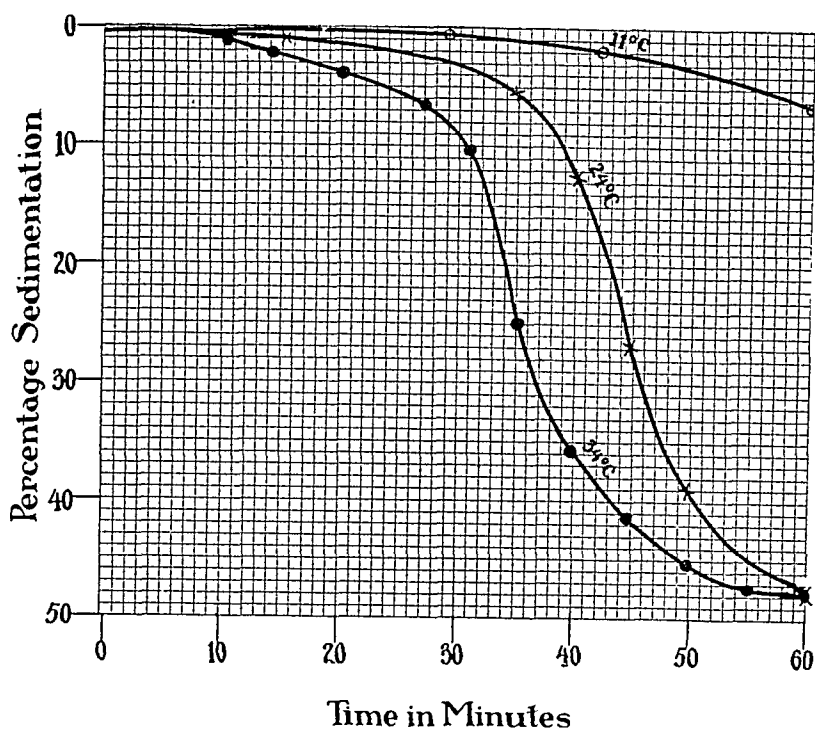


Fig. 2.—Influence of temperature on the sedimentation of red blood corpuscles.

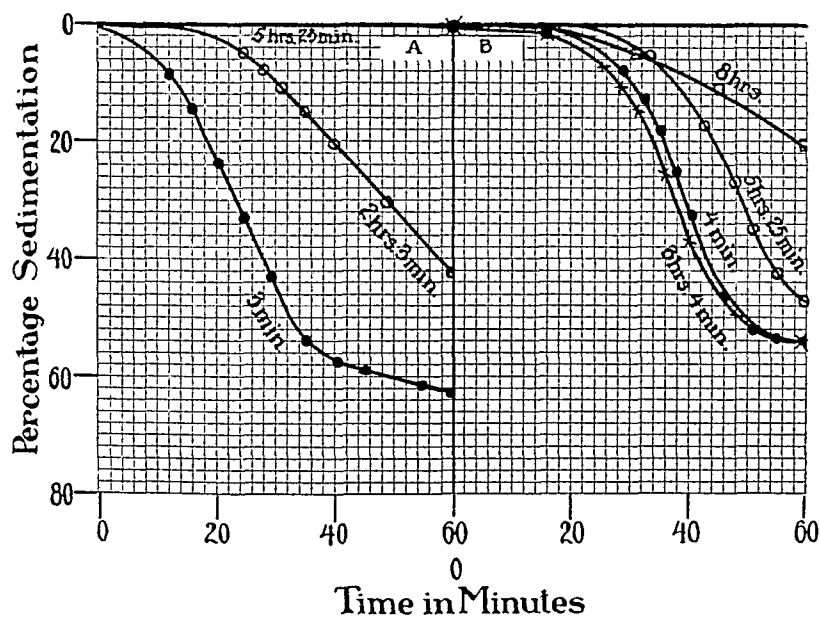


Fig. 3.—Influence of delay on the sedimentation of red blood corpuscles. A, Potassium oxalate used as anticoagulant. B, Heparin used as anticoagulant.

to each end of the rod after passing it through the center of the metal spring. A drop of acid is placed on the spring and rod and a rosin cored solder is used. The advantages of using these clips are

- 1 It provides a perfect means of holding the paper during the shellacking
- 2 The clips prevent the paper from curling while drying
- 3 The bottom clip has sufficient weight to prevent gusts of air blowing the wet papers and causing the adjacent tracings to stick together

After placing the clips on each end of the paper it is run through the shellacking tank. The shellacking tanks are connected by rubber tubes to aspirator bottles of one gallon capacity. To fill the tanks the bottles are placed on a shelf above the tank, thus causing the fluid to run in by gravity. When not in use, the bottles are placed on a shelf below the tank and the shellac flows back into the bottle, preventing undue evaporation of the solvent. We dilute one part of ordinary commercial shellac with three parts of alcohol, allow it to settle and drain off the clear supernatant fluid. This gives a thin, quick drying shellac which perfectly protects the tracings.

When the record has been shellacked and the excess fluid drained off it is hung up to dry. The drying racks are made by boring holes in the tops of ordinary spring clothespins and stringing them on picture hanging wire. This wire is stretched taut at such a height as will permit the record to hang free. The clip on the wire is fastened to the middle of the brass rod of one of the paper holding clips.

It is advisable to place a drip pan underneath the drying rack, although this is not necessary if the students are made to thoroughly drain their tracings over the shellac tank before hanging them up to dry.

## AN INEXPENSIVE IMPROVED SHAKING MACHINE\*

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IN EVERY pathology laboratory associated with a large hospital, there is urgent need for an oscillating or "shaking" machine. This is especially true now, since the precipitation tests for syphilis have come into vogue. Thus, in performing the Kline test a shaking machine is almost indispensable. Such a device, in addition to conserving time and the energy of technicians, serves the additional purpose of standardizing technics in the various tests in which the degree of admixture of the interacting elements constitutes a factor in the final reading.

The most serious obstacle toward the ownership of such a machine is its cost, which is about sixty to seventy-five dollars. Since the proverbial poverty

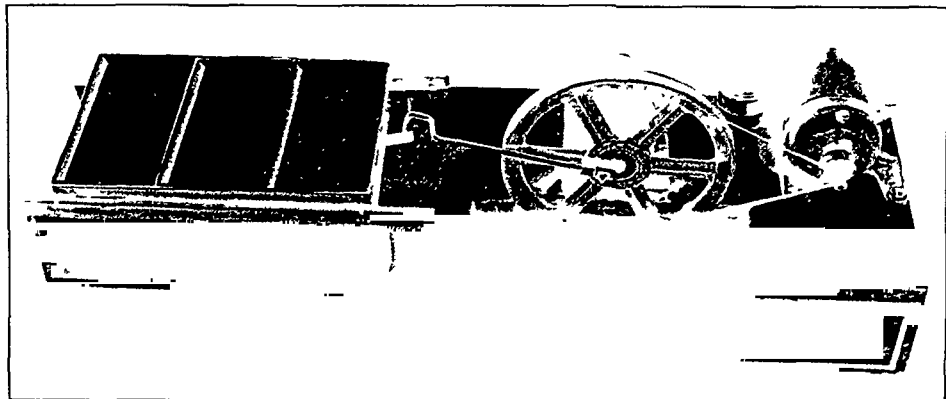


Fig. 1.—Photograph of oscillating machine.

of the average pathology department would prohibit such an expenditure, it is frequently necessary to resort to "home talent" which was done in the present case, with the result that an efficient oscillator was designed and constructed at a cost of approximately three dollars.

Briefly, the apparatus consists of a wooden platform on which is mounted a small  $1/32$  H. P. universal motor connected by a belt to a flywheel. The hub of the flywheel bears an eccentric rod which attaches to a shallow tray fixed upon metal runners sliding in metal tracks. The rapid revolutions of the motor are reduced to a lower speed by the flywheel, and through the medium of the eccentric rod, the rotary motion is converted into horizontal to-and-fro movement of the tray holding the slides or tubes. Careful observation of the rate and amplitude at which the laboratory technicians were accustomed to shake the Kline slides by hand before "reading" them revealed it to be 200

\*From the Department of Pathology, Cumberland Hospital.  
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## FALSE NEGATIVE OR POSITIVE REACTIONS

1 *Negative Reactions*—If the specimen is obtained in less than ten days following the first missed period, the pituitary hormone may or may not be excreted in sufficiently large quantities. Then, there is at times a blockage of the hormone to the kidney which may occur even a month following suspected conception. In both cases a positive report may not be rendered.

2 *Positive Reactions*—It is generally supposed that the urine of patients having certain types of tumors (chorionic) may contain gonad stimulating hormones, thus rendering a Friedman test positive. Even two months after removal or expulsion of the tumor a positive reaction may persist.

## HOFFMAN'S MODIFICATION

In this modification blood serum is used. This necessitates drawing about 25 cc of blood which may be taken at any time of the day. The rabbit may be autopsied within twenty-four hours, probably due to the fact that there is a larger amount of anterior hypophyseal hormone in the blood during earlier pregnancy. Too, only rarely will the reaction of the blood need adjustment.

*Technic*—Centrifuge blood specimen, and shake with ether to remove fatty substances, inject 10 to 13 cc of the serum into the marginal ear vein of the rabbit.

It is hoped that this modification will supplant that in which urine is used, at least, perhaps, until some quicker, more efficient technic in early pregnancy diagnosis will be found.

## REFERENCES

- 1 Kolmer and Boerner. Approved Laboratory Technic, Chap XXXVI, p. 611
- 2 Dimmitt, P. S. Clinical Laboratory Methods, Philadelphia, 1934, F. A. Davis Co., Chap XVII, pp. 139 and 140
- 3 Zentralbl f. Gynak., Oct. 15, 1932

350 POST STREET

to 300 times per minute through a horizontal distance of about one inch. Consequently, in order to obtain the proper rate of oscillation of the slide tray, it was necessary to gear down the speed (R.P.M.) of the motor. This was done by selecting a motor with a  $\frac{1}{4}$  inch shaft, on which was fastened a  $\frac{3}{8}$  inch pulley wheel. The diameter of the flywheel employed obviously depends upon the R.P.M. of the motor. This diameter may be determined by applying the following approximation:

$$\frac{\text{R.P.M. of motor}}{\text{Desired rate of oscillation (200)}} = \frac{\text{Diameter of flywheel (X)}}{\text{Diameter of motor pulley } (\frac{3}{8} \text{ inch})}$$

Once the proper flywheel is obtained, it is suspended from one side by a sturdy support and an eccentric rod is connected to the hub at a distance of  $\frac{1}{2}$  inch from the exact center of the wheel, thus imparting a one-inch horizontal oscillation to the attached slide-tray with each complete revolution of the flywheel. The tray for holding the slides or tubes resembles a sleigh, consisting as it does of a shallow box on metal runners. The latter are confined to well-oiled tracks composed of U-shaped metal strips which are fastened by their sides to wooden platforms. If it is desired to vary the speed of oscillation, a small wire or graphite variable resistance, such as those used for radio hookups, may be interposed in the circuit between the switch and the motor. Finally a coat of paint completes the job.

All the parts required for this apparatus are obtainable at small cost at any machine or machinists' supply shop and, with the aid of the hospital engineer or machinist, can be assembled with very little effort. The resulting oscillating machine is sturdy, practical, and adaptable to the innumerable uses which daily laboratory routine requires.

maximum rate, and they are strung out, making it difficult to read the columns accurately. The average angle made by the sedimentation curve and the horizontal, during the second phase (maximum speed of sedimentation), was  $69^\circ$  with a variation from  $67^\circ$  to  $70^\circ$ . The results demonstrate reproducibility of the curves.

The influence of changes in temperature is illustrated in Fig. 2. At  $11^\circ \text{C}$ . there is a remarkably slow settling of the red blood corpuscles compared with

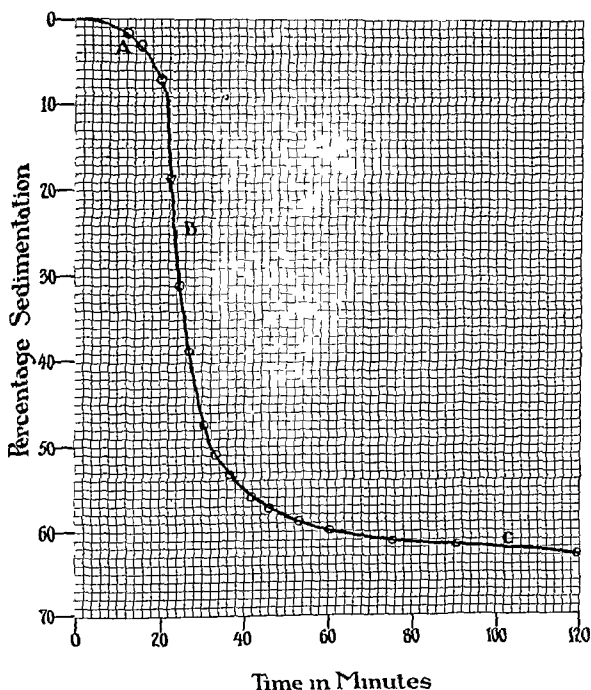


Fig. 1.—Typical red blood corpuscle sedimentation curve.

that at  $24^\circ \text{C}$ . At  $34^\circ \text{C}$ ., during the first phase there is a marked increase in speed, shortening the period of induction. During the second phase there is a slight increase in the speed from that of the  $24^\circ \text{C}$ . curve.

Fig. 3 shows the influence of delay in performing the sedimentation test. For this experiment 4 c.c. of venous dog blood were drawn into a dry syringe. Two cubic centimeters of this blood were mixed with 2 drops of heparin solution, and the other 2 c.c. of blood were mixed with 2 drops of potassium oxalate solution. The sedimentation rate was tested immediately; the blood was then

After spraying had been accomplished the chamber was blown out with air. Then the mouse was removed, a small cotton plug wrung out in 5 per cent lysol was placed over the diaphragm, the snout of the animal wiped off with alcohol, and the animal placed with others of the series in a wire cage. When an entire series was completed, the whole apparatus was quickly dropped into a container of boiling water kept at hand.

Before the device was used its air-tightness was tested by placing a dead mouse at the diaphragm and submerging the whole in water. It was found to be perfectly air-tight when operated either by the hand-bulb or by an air-line at low or higher pressure.

For our first experiments the mice were anesthetized with nembatal. But later, it was found possible to work with no anesthetic at all. In the latter case, the forefeet of the animal were tied, or clamped together with tongue

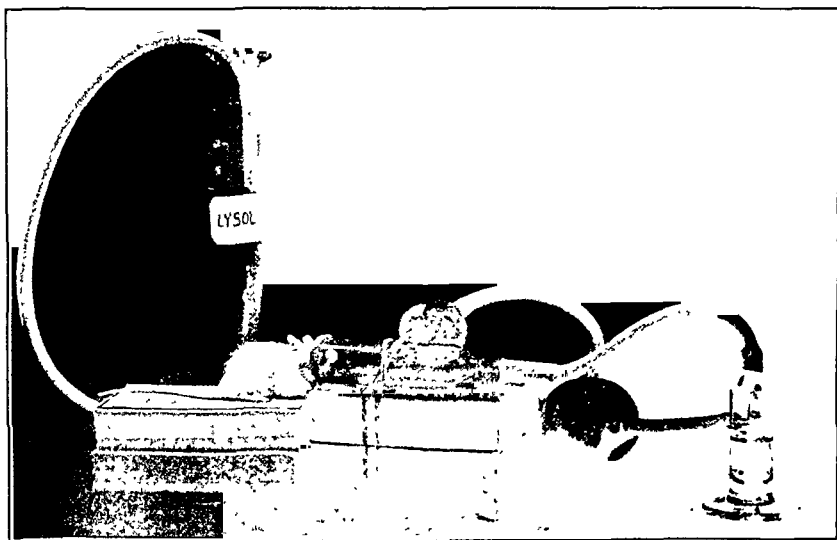


Fig. 1.

forceps, and the animal held firmly in place by the skin at the back of the head. If no anesthetic is used, care must be exercised not to permit the animal to bite the rubber diaphragm as the snout is inserted. However, once in position, our mice usually offered little resistance and often could be left unattended for several minutes.

The efficacy of the apparatus described was tested with the virus of St. Louis encephalitis, and also with Friedländer's bacillus. In the case of the virus, 5 test groups of 5 mice each were tested, all the animals used being of the ordinary white laboratory variety and weighing approximately 20 gm. A 2 per cent virus suspension by weight, made by triturating one whole infected mouse brain without abrasive in a mortar, adding diluent and centrifuging, was used. For the first test the suspending fluid employed was nutrient broth. Later twice filtered (through Berkefeld V and W candles) human saliva, which had proved negative for bacterial or viral growth by cultivation and animal

allowed to stand and the sedimentation test was again made at intervals as indicated in Fig 3. The results show first, delay causes slowing of sedimentation rate with both oxalated and heparinized blood. Second the effects of delay are shown much earlier with oxalated blood than with heparinized blood, and third, heparinized blood settles more slowly than oxalated blood.

Fig 4 shows the great acceleration of the sedimentation rate resulting from a slight deviation of the pipette from the vertical position. A change in the angle of the sedimentation pipette from  $0^\circ$  (perpendicular position) to  $5^\circ$  causes a change in the maximum fall of the curve from a  $35^\circ$  angle to a  $63^\circ$  angle. At a  $15^\circ$  tilt in the pipette the maximum fall in the curve made a  $74^\circ$  angle with the horizontal.

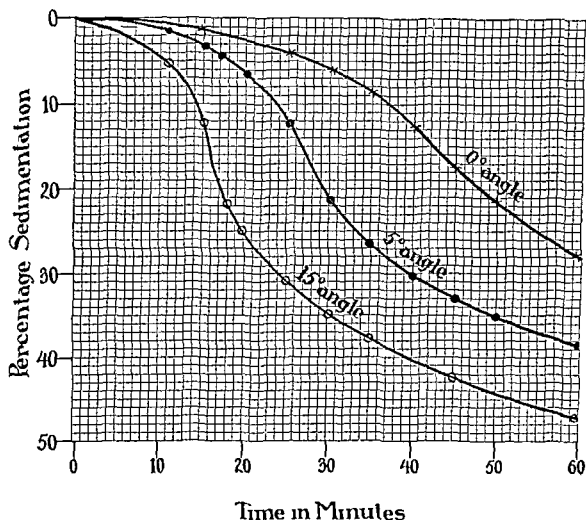


Fig 4—Influence of change in angle of pipette from the perpendicular on the sedimentation of red blood corpuscles.

#### SUMMARY

The results of the new *micro* red blood corpuscle sedimentation technique previously devised by one of us are satisfactorily reproducible. The rate of red blood corpuscle sedimentation is increased with increase in temperature and by tilting the sedimentation pipette. Delay after drawing the blood causes a slowing of the sedimentation rate. This is true for both oxalated and heparinized blood.

#### REFERENCE

- 1 Brooks, Clyde. *J LAB & CLIN MED* 21: 971, 1936

# AN IMPROVED CAPSULE FOR ASCERTAINING VENOUS PRESSURE\*

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THE glass,<sup>1</sup> metal,<sup>2</sup> and celluloid<sup>3</sup> capsules previously devised for the indirect ascertainment of venous pressure have undesirable characteristics, among which are the lack of adaptability and difficulty of sealing, which handicap their routine use. The rubber-celluloid capsule described below eliminates many of these shortcomings. Air-tight skin contacts can easily and quickly be made over smooth or irregular surfaces which will withstand pressures up to 100 mm. of Hg with a minimum of skin distortion and no abnormal pressure on the vein. Its construction is simple and inexpensive and the same capsule can be used repeatedly without repair. No special lighting is required and readings are easily made which agree within 1 cm. of water with simultaneous direct puncture measurements.

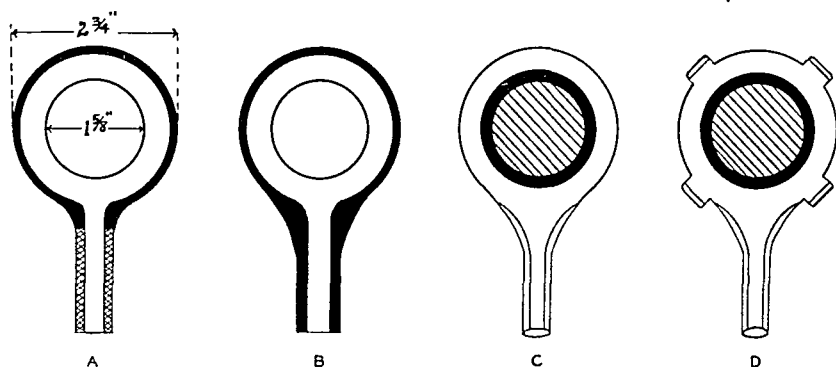


Fig. 1.—A, Inner surface of top disc; B, inner surface of bottom disc; C, upper surface of complete capsule; D, complete capsule with projections for strap attachments. The dimensions given above have been found most suitable for the average hand. Solid black represents cemented surface; cross-hatched represents cemented surface on opposite side; diagonally-lined represents celluloid.

The capsule is constructed as follows: Two washer-like discs of rubber (bicycle inner tubing) with extensions, as shown in Fig. 1A and B, are roughened and cemented together on their margins as indicated. The edges of the wider extension of B are brought up and around the narrower one of A and cemented to form the side tube as shown in C. A disc of celluloid  $1\frac{7}{8}$  inches in diameter is roughened on its margins and cemented over the opening in the upper disc. All of the outer surface of the lower disc is roughened for cementing the capsule to the skin. Regular cold-patch rubber cement is used for assembling the capsule and sealing it to the skin. Both the capsule and the skin are coated with cement and allowed to dry before putting the capsule in place. Because of the large surface for making the skin-capsule seal, skin

\*From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana. Received for publication, April 27, 1936.



animals to reach through and touch the metal channel pieces. The sides of each individual cage are glass plates 6 by  $8\frac{1}{4}$  inches set off from the intermediate metal baffles and end metal plates approximately 15 mm. in order to prevent the animal from coming in contact with them. The glass plates are held in place by sliding them between 7 mm. glass tubes supported in the horizontal channel pieces. They are prevented from falling through the bottom of the cage by resting on 10 mm. glass tubes supported in the bottom horizontal channel pieces. The cover of each cage is a glass plate  $9\frac{5}{8}$  by  $7\frac{5}{8}$  inches which rests on the glass plate sides and top horizontal tubes. It is held in place by the metal frame of the cage unit, and the animal is kept from pushing it up, either by the top of the rack shelf into which the frame slides, or by weighting.

When the glass tubes have all been installed, they are secured on the ends of the cage by flanged metal cover plates, and on the sides by slightly smaller channel strips which slide into and fasten to the horizontal strips.

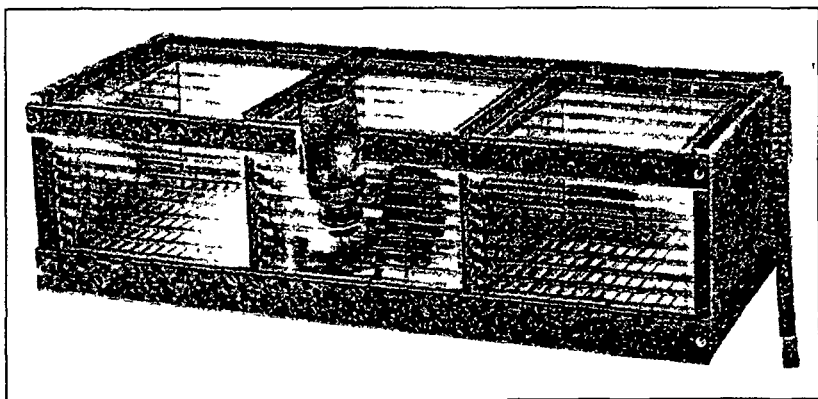


Fig. 1.

This type of construction has the particular advantages that none of the glass parts are held rigidly, no ends are exposed, and it can be easily sterilized. Enough play is allowed so that no breakage can result from strains due to slight distortion of the metal frame. To allow for variations in tube sizes, all holes are drilled approximately 0.5 mm. larger than the tube desired and if breakage does occur, replacements are easy.

For collection of urine and feces, Pyrex baking dishes,  $8\frac{1}{2}$  by 11 inches, are placed under each cage. When it is desired to separate urine and feces, a grating of glass tubing, similar in construction to that of the floor of the cage, held in a frame somewhat larger than the dish, and resting directly on it, will serve. Water bottles for each cage are held in place by a metal spring clamp secured to the top horizontal channel on one side.

#### REFERENCES

1. Nevens, W. B., and Shaw, D. D.: Nutritional Anemia in the White Rat, *Science* 72: 249, 1930.
2. Underhill, F. A., Orten, J. M., and Lewis, R. C.: Inability of Metals Other Than Copper to Supplement Iron in Curing Nutritional Anemia of Rats, *J. Biol. Chem.* 91: 13, 1931.

# AN APPARATUS FOR SPRAYING THE NASAL PASSAGES OF MICE\*

## EXPERIMENTS WITH ST LOUIS ENCEPHALITIS VIRUS AND FRIEDLANDER'S BACILLUS

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**T**HAT mice may be successfully infected with the virus of St. Louis encephalitis when inoculated by the intranasal route was demonstrated originally by Webster and Fite,<sup>1</sup> and confirmed by Armstrong,<sup>2</sup> Brodie,<sup>3</sup> and other workers. The favorite method of instillation is by dropping a small quantity of the material from a syringe directly into the nares of the anesthetized animal. Other methods that have been employed are insertion of the needle of the syringe into the external nasal passages with release of the inoculum and forced inhalation by holding the mouth and nose of the animal in a container of the infectious substance for a few seconds.

In the course of our experiments with this virus, we wished to develop a technic of intranasal inoculation that would simulate more closely natural infection and yet would involve a minimum of risk to the worker. Inhalation of material suspended in sterile saliva and worked into a mist of fine droplets seemed to satisfy the first requirement. While such a technic has not as yet, to our knowledge, been used in the inoculation of mice with the virus of St. Louis encephalitis, it has been employed in studies of various bacteria, especially the pneumonia-inciting group. Friedländer,<sup>4</sup> Wherry and Butterfield,<sup>5</sup> Stillman,<sup>6</sup> Griffith<sup>7</sup> and others have described apparatuses for spraying mice with diverse bacterial suspensions. The general principle involved in each case has been the use of a hand spray connected to a chamber in which the mice were confined. The greatest disadvantages of such an arrangement are the danger in opening the chamber to remove the mice and the fact that the entire surface of the animal's body is contaminated with the inoculum.

To avoid these factors we devised the mechanical arrangement shown in Fig. 1. The apparatus consists of a small tubular glass chamber, open at one end and containing two outlets. To one outlet, a nebulizer is securely connected by means of rubber tubing. The other outlet, designed with a glass bulb for equalization of pressure, is loosely plugged with cotton and connected with a very long rubber tube suspended at the free end over lysol solution in a tall glass cylinder. Over the open end of the chamber is tightly fitted a rubber diaphragm in which a small puncture hole is made to admit the snout of the rodent.

In the experiments recorded, the spray was produced by means of a strong rubber bulb connected to the nebulizer. However if convenient an air-line regulated to very low pressure may be employed.

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*Bacteriologists* (ed. 5, lv, 34-9). This modification is particularly valuable in staining *Treponema microdentum* in smears taken from the oral cavity or throat. Likewise, *Borrelia vincenti* and *Treponema macrodentum* take a clearer and deeper stain, differentiating them from their surroundings and making it possible to demonstrate distinctive characteristics so important in the study of spirochaetaceae.

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## ACETONE SOLVENTS FOR ROMANOWSKY STAINS\*

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### INTRODUCTION

ACCORDING to modern authorities on staining technic, the use of acetone as a solvent for Romanowsky stains is to be avoided. The two types of stain most utilized, represented by Wright's (1902; 1910) and Giemsa's (1904; 1907), both contain methyl alcohol. MacNeal (1922; 1925), whose tetrachrome stain has been considered one of the most scientific yet devised for blood, likewise uses methyl alcohol as a solvent.

Directions for preparing any of these stains contain such admonitions as these:

"... Purchase the powders . . . and dissolve 0.3 gram in 100 c.c. of special acetone-free methyl alcohol." (Kolmer, Boerner and Garber, 1931, for the American Society of Clinical Pathologists.)

"If the stain is prepared . . . by dissolving the powdered stain in alcohol it is necessary to use glassware chemically clean and methyl alcohol for staining purposes that is acetone-free." (Nicholson, 1930.)

Conn (1929) in a general review of modern knowledge of the chemistry of Romanowsky staining, counsels:

As stated above, the precipitated compound dye must be dissolved in methyl alcohol; but there are many grades of methyl alcohol and not all are equally suitable for the purpose. Apparently absolute purity is not needed; but two points are very important; the methyl alcohol must be neutral in reaction, and it must be free from acetone.

Stains used at present require a technic in which so many variable factors coexist that the action of the solvent does not lend itself readily to investigation. In a series of over 260 experiments on Romanowsky stains, a systematic study of solvents was conducted. Some of the findings indicated that a more volatile fluid might have certain advantages over methyl alcohol. Among the liquids tried, acetone was discovered to be entirely suitable. On the whole, it proved superior to methyl alcohol as a solvent for Romanowsky stains.

Its use was therefore proposed in a stain (Kingsley, 1935) which is satisfactory not only for blood smears but also for most types of hemopoietic tissue preparations, including frozen sections. Furthermore, since the final

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passage, was substituted. Saliva may be obtained in large quantities, and was found to preserve the St. Louis virus in higher titer than either broth or physiologic saline solution of the same pH. About 1 cc of the material was sprayed into the chamber and the rodent allowed to breathe thereof for five minutes. Sixty-eight per cent of the mice so treated developed the typical symptoms of this form of encephalitis: tremors, convulsions, prostration, with fatal outcome in six to eleven days. Microscopic examination of preserved brain tissue revealed the perivascular accumulation of round cells and nerve cell degeneration characteristic of the disease.

Fifteen white mice in all in 3 series of 5 mice each were inoculated with a very heavy suspension of Friedlander's bacillus in sterile saliva. These mice showed an average mortality of 73.3 per cent. The low fatality (40 per cent) for the last test may possibly be attributed to the fact that the strain used had been kept for some time on artificial media, whereas that of the other 2 tests had undergone recent animal passage.

The detailed results for these two series of tests are incorporated in Table I.

TABLE I  
RESULTS OF INHALATION OF ST. LOUIS VIRUS AND FRIEDLANDER'S BACILLUS BY MICE

INOCULUM	NO. OF MICE IN TEST GROUP	ANESTHETIC	SUSPENDING MEDIUM	DEATHS DUE TO INOCULUM	DAY OF DEATH	PERCENTAGE MORTALITY
Virus of St. Louis encephalitis	5	Nembutal	Broth	2	2, 8, 11	40
	5	None	Saliva	4	7, 7, 7, 9	80
	5	None	Saliva	3	6, 7, 9	60
	5	None	Saliva	4	6, 7, 7, 7	80
	5	None	Saliva	4	7, 7, 7, 8	80
Total	25			17		68
Friedlander's bacillus	5	None	Saliva	0	2, 2, 2, 3, 3	100
	5	None	Saliva	4	2, 2, 3, 3	80
	5	None	Saliva	2	4, 5	40
Total	15			11		73.3

\*Found dead and partially devoured.

#### SUMMARY

An apparatus for spraying the nasal passages of mice is described. The device has been found safe and has been used successfully with suspensions of the virus of St. Louis encephalitis and of Friedlander's bacillus.

#### REFERENCES

1. Webster, L. T., and Fite, G. L. A Virus Encountered in the Study of Material From Cases of Encephalitis in the St. Louis and Kansas City Epidemics of 1933, *Science* 78: 463, 1933.
2. Armstrong, C. The Production of Specific Immunity in White Mice by Intranasal Inoculation With Encephalitis Virus (St. Louis Type), *Pub. Health Rep.* 49: 959, 1934.
3. Brodie, M. Route of Transmission of St. Louis Encephalitis Virus in Mice, *Proc. Soc. Exper. Biol. & Med.* 32: 1647, 1935.
4. Friedlander, C. Die Mikrokokken der Pneumonie, *Fortschr. d. Med.* 1: 715, 1887.
5. Wherry, Wm. B., and Butterfield, C. T. Inhalation Experiments on Influenza and Pneumonia, *J. Infect. Dis.* 27: 315, 1920.
6. Stillman, E. G. The Presence of Bacteria in the Lungs of Mice Following Inhalation, *J. Exper. Med.* 38: 117, 1923.
7. Griffith, F. Inhalation Experiments on Mice With Pneumococci, *J. Hyg.* 25: 1, 1926.

(equal parts of methylene blue and azure A), and the other, eosin. The actively staining substances were formed only after mixing these two solutions. The preparations required previous fixation, as in all technics in which the dye solvents are not likewise fixatives.

Although Giemsa was acquainted with the advantages of Leishman's method, he did not find methyl alcohol a suitable solvent until he added to it an equal part of glycerin. Without the glycerin, Giemsa (1902 b) had previously been unable to keep the purified azure-eosin in solution, even though he used the purest, acetone-free methyl alcohol. This is his only statement indicating that acetone might in some way be detrimental for blood staining. But the inference is more probable that acetone-free alcohol designated a pure grade of alcohol, rather than that acetone itself is harmful. This conclusion is substantiated by more recent statements of Giemsa (1935). In 1904 Giemsa simplified his stain by dissolving the powdered azure II eosin in equal weights of methyl alcohol and glycerin, a ratio which was changed to three parts of alcohol to one of glycerin in 1907.

The only other stain to be presented was that of MacNeal (1922; 1925), although he offered nothing new in regard to the solvents. His studies were directed, quite successfully, toward the problem of the dyes necessary for Romanowsky staining and led to the tetrachrome stain, which is used in methyl alcohol like Wright's. Only in his most recent formula does MacNeal (1925) specify acetone-free methyl alcohol as the solvent.

Actual references to the necessity for using acetone-free methyl alcohol are therefore extremely few and seem entirely incidental, with no particular emphasis laid on this point by the originators of the various methods. The textbook authors have apparently stressed this point unduly. On the other hand, there are in the literature several positive statements about the advantages of acetone in the solvent.

Michaelis (1899) was the first to advocate the use of acetone in a blood stain. His studies resulted in a stain which did not give Romanowsky effects, but the solvent contained 35 per cent acetone. He thought that acetone prevented precipitation of the dyes and facilitated the staining of the neutrophilic granules. Later, Michaelis (1901 a) recommended a stain much like Jenner's (1899). Very soon after, however, Michaelis became cognizant of Bernthsen's (1885) work in the chemistry of the basic dyes necessary for Romanowsky stains, and applied it (1901 b) immediately to the production of a new stain, in an aqueous solvent.

While Giemsa (1902 b) mentioned acetone at first in only a derogatory way, he utilized it later (1910) when he advocated a rapid technic similar to Leishman's. In this method he fixed with his stock solution diluted one-half with methyl alcohol, then stained by adding water. Tucked away at the end of his article, he made this very significant statement, p. 2476:

One can also dilute the stock solution with acetone (puriss., Merck or Kahlbaum) instead of methyl alcohol. The various plasma granules are then especially well brought out. However, because of its low boiling point (56°), acetone is hardly to be recommended for use in warm climates.

distortion is reduced to a minimum. By virtue of the same characteristic plus the flexibility of the capsule and congruity of the two discs rapid and perfect sealing is obtained in about thirty seconds.

The modified Capsule D is used where extremely high pressures are encountered, such as those in the foot with the body in the upright position (1,000-1,200 mm H<sub>2</sub>O). The straps are joined snugly around the foot and become tight at the high pressures and prevent the capsule and skin from moving away from the vein and thus falsely giving it the appearance of being collapsed. Krogh and others<sup>3</sup> developed a calibrated spring clamp for this purpose when using an all celluloid capsule.

#### REFERENCES

- 1 Hooker, D. R. Venous Blood Pressure, *Am J Physiol* 40: 43, 1916.
- 2 Eyster, J. H. E. The Clinical Aspects of Venous Pressure. New York 1929, The Macmillan Co., p. 22.
- 3 Krogh, A., Turner, A. H., and Landis, I. M. Celluloid Capsule for Measuring Venous Pressure, *J Clin Investigation* 11: 357, 1932.

### AN IMPROVED GLASS METABOLISM CAGE FOR SMALL ANIMALS\*

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**D**URING the course of our experiments it was found necessary to keep the animals out of contact with metal. Two types of glass cages have been described in the literature,<sup>1, 2</sup> both of which depended on a wooden frame to support the glass parts. Such wooden frames, while light, are easily contaminated with urine and feces, and offer harboring places for vermin. Furthermore, they are subject to warping, with possible breakage of glass parts when heat sterilized. We have, therefore, designed a light metal framework which may be copper, aluminum, or galvanized iron, suitable for supporting one to three unit cages, 8¼ inches long, 6½ inches wide, and 5½ inches high. The construction of a three unit cage is illustrated in Fig. 1.

The metal frame consists of four horizontal channel strips, each 2¼ inches long (1 inch wide for the top, and 1½ inches wide for the bottom) riveted to two flanged end plates and two intermediate baffles, 9¾ by 7 inches. The two bottom horizontal channel strips are drilled with holes 12.5 mm from center to center and large enough to hold 7 mm glass tubing for the floor of the cage. This gives a spacing of 5.5 mm between the glass tubes sufficient to allow feces to drop through. A vertical row of holes spaced 13 mm from center to center is drilled approximately 15 mm from each side edge of the end plates and intermediate baffles. These are large enough to hold 5 mm glass tubing on the horizontal for the front and back walls. This gives a spacing of 8 mm between the glass tubes. The fact that the horizontal tubes are placed 15 mm from the edges of the plates makes it impossible for the

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## EXPERIMENTS

The experiments to be described are part of a large series undertaken to determine systematically the dyes requisite for Romanowsky staining, the rôle of each in the production of a given color effect, and the relationship of the solvent to the final results. A full report will appear in another paper. At this time, only a few examples of those experiments in which acetone solvents were used successfully will be described.

Attempts were first directed to the production of a single staining solution which would be stable and always ready to use by merely pouring it onto a fixed slide. This single solution contained water, a buffer, and methyl alcohol, as solvents for the necessary dyes. Among other observations, it was noticed that many stains which were poor in general, were quite satisfactory at the edges of the slides. In this region of the preparation, the staining fluid differed in no respect from that anywhere else on the slide, since a single fluid had been prepared previously. Physically, however, conditions at the edges of the slide are different from those existing away from the borders, because the depth of the fluid layer is less at the margins than elsewhere. This was noted to result in the formation of a metallic-appearing precipitate (consisting of the eosinates of methylene blue, azure A, and violet), considerably earlier at the edges than anywhere else on the slide. This condition seemed to be a result of the thinness of the fluid layer at this region, which facilitated the evaporation of the methyl alcohol and left a relatively high water concentration in this region.

On the basis of this reasoning, a fluid which would be more volatile than methyl alcohol should allow a lower initial concentration of dyes to be present, thus decreasing the tendency to precipitation when water is present; yet, by rapid evaporation, a satisfactory dye concentration should be obtained.

Acetone seemed to possess the desired physical attributes and was therefore tried, with excellent results. Several formulas follow. In these, the dyes are listed in grams and the solvents in cubic centimeters per 100 c.c. of final stain.

It is possible to prepare a good stain which contains no methyl alcohol whatsoever, but has acetone instead, as in the following stain:

Acetone	60 c.c.
Glycerin	5 c.c.
Buffer pH 6.9	35 c.c.
Methylene blue	0.040 gm.
Methylene azure A	0.015 gm.
Methylene violet	0.007 gm.
Eosin, yel.	0.040 gm.

A very rapid, excellent stain is prepared by the following formula:

SOLUTION A		SOLUTION B	
Water	40 c.c.	Acetone	35 c.c.
Buffer pH 6.9	10 c.c.	CH <sub>3</sub> OH	10 c.c.
Methylene blue	0.070 gm.	Glycerin	5 c.c.
Methylene azure A	0.025 gm.	Methylene violet	0.018 gm.
		Eosin, yel.	0.065 gm.

Mix equal volumes of A and B to obtain the stain.

## THE USE OF COLLOIDAL IODINE AS A MODIFICATION OF THE GRAM STAIN\*

DON CHALMERS LYONS M S, D D S P H D, JACKSON, MICH

THE usual Gram stain consisting in the use of aniline gentian violet or carbol gentian violet, iodine solution, ethyl alcohol, and a counterstain such as eosin is satisfactory in most instances. Many microorganisms, however, do not stain readily, particularly in the presence of organic material such as one finds in thick smears taken from body effusions and especially those from the mouth.

Gram's modification of Lugol's solution consisting of sublimed iodine (1 gm), potassium iodide (2 gm), distilled water (300 cc), does not have sufficient penetrating power to completely fix the cell walls of many types of microorganisms or their morphologic variants. This may be due to the fact that the solvent KI does not readily give up iodine to the microorganism.

In order to bring about a reaction between the bacteria and the stain, iodine must be in the most active state, therefore the ideal solution is a constant water solution. Iodine in the form of sublimed crystals goes into solution in water very slowly, requiring days or even weeks to produce a saturated water solution.

Water at room temperature dissolves elemental iodine to the extent of only about 3 parts in 10,000 and being a poor solvent, readily releases iodine to organic matter. For this reason colloidal iodine is an ideal source of iodine for the Gram stain because no solvent stronger than water is present. It is a finely divided pure iodine held in suspension in water in excess of the point of saturation. Pure iodine in this state can replace iodine lost from the solution, thus keeping the solution constantly saturated, so that in this modification of the Gram stain the suspended particles constantly replace the iodine taken from the solution by organic matter. This permits a complete satisfaction of the iodine fixing powers of the microorganisms.

The solubility of colloidal iodine as compared to the crystalline form is readily understandable. A saturated water solution of iodine ordinarily requires several days for formation from ordinary crystalline iodine, due to the slight solubility of iodine (0.03 per cent at 20° C). In the case of colloidal iodine the production of a saturated solution is instantaneous†.

A 1 per cent suspension of colloidal iodine is prepared and substituted for the usual Gram's iodine solution in the routine of this staining procedure as outlined in the *Manual of Pure Culture Study of the Society of the American*

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† Chandler W. L. and Miller F. L. Colloidal Iodine J. Phys. Chem. 31 1091 1927

ordinary methyl alcohol solution of Wright's blood stain, and an extra minute or two allowed for staining to compensate for the dilution of the stain, excellent stains were obtained.

#### CONCLUSIONS

Difficulties which have not previously been analyzed have led to erroneous conclusions concerning the constituents of Romanowsky stains. Among others, acetone has been included as a detrimental substance. Actually, as the present report demonstrates, acetone is useful as a solvent for blood stains, and in some respects is superior to methyl alcohol. With a given dye concentration, it permits more rapid staining than does methyl alcohol. In addition, it is a better solvent for methylene violet, which is a necessary constituent of any good Romanowsky stain.

The purest grade of methyl alcohol is also acetone-free. But experiments on the participation of solvents in the staining process indicate that the concentration of water is the most important variable in the solvent, and not the presence of acetone. Indeed, in the stain advocated as best for general use, acetone constitutes most of the nonaqueous portion of the solvent.

#### REFERENCES

- Bernthsen, A.: Studien in der Methylenblaugruppe, *Ann. Chemie* 230: 73, 1885.
- Carageorgiadès, H.: Deux colorants neutres pour la méthode panoptique de préparation facile et rapide, *Compt. rend. Soc. de biol.* 81: 925, 1918.
- Conn, H. J.: Biological Stains. A Handbook on the Nature and Uses of the Dyes Employed in the Biological Laboratory, ed. 2, 1929. Commission on Standardization of Biological Stains, Geneva, New York.
- Giemsa, G.: Färbemethoden für Malariaiparasiten, *Centralbl. f. Bakt., I. Abt.* 31: 429, 1902 a.
- Giemsa, G.: Färbemethoden für Malariaiparasiten, *Ibid.* 32: 307, 1902 b.
- Giemsa, G.: Eine Vereinfachung und Vervollkommen meiner Methylenblau-Eosin-Färbemethode zur Erzielung der Romanowsky-Nochtschen Chromatin-färbung, *Ibid.* 37: 308, 1904.
- Giemsa, G.: Beitrag zur Färbung der *Spirochäte pallida* (Schaudinn) in Austriech-präparaten, *Deutsches med. Wehnschr.* 33: 676, 1907.
- Giemsa, G.: Ueber eine neue Schnellfärbung mit meiner Azureosin-lösung, *München. med. Wehnschr.* 57: 2476, 1910.
- Giemsa, G.: Ueber eine bemerkenswerte Fehlerquelle bei der färberischen Darstellung der Schüffner-Tüpfelung, *Ibid.* 82: 1075, 1935.
- Jagic, N.: Ueber Azetonfixierung von Blutpräparaten, *Wien. klin. Wehnschr.* 19: 587, 1906.
- Jenner, L.: A New Preparation for Rapidly Fixing and Staining Blood, *Lancet* 1: 370, 1899.
- Jonesco-Mihaiesti, C.: Nouvelle formule d'une solution panchromatique pour la coloration du sang et des Protozoaires, *Compt. rend. Soc. de biol.* 81: 1088, 1918.
- Kingsley, D. M.: A New Hematological Stain. I. Constituents and Methods of Use, *Stain Techn.* 10: 127, 1935.
- Kolmer, J. A., Boerner, F., and Garber, C. Z.: Approved Laboratory Technic, *Am. Soc. Clin. Path., Appleton*, 1931.
- Leishman, W. B.: A Simple and Rapid Method of Producing Romanowsky Staining in Malarial and Other Blood Films, *Brit. M. J.* 2: 757, 1901.
- Leishman, W. B.: Note to article by Tulloch, 1904.
- MacNeal, W. J.: Tetrachrome Blood Stain: An Economical and Satisfactory Imitation of Leishman's Stain, *J. A. M. A.* 78: 1122, 1922.
- MacNeal, W. J.: Methylene Violet and Methylene Azure A and B, *J. Infect. Dis.* 36: 538, 1925.
- Malachowski, E.: Zur Morphologie des Plasmodium malariae, *Centralbl. f. klin. Med.* 12: 601, 1891.
- Michaelis, L.: Eine Universalfärbemethode für Blutpräparate, *Deutsches med. Wehnschr.* 25: 490, 1899.
- Michaelis, L.: Ueber die Methylenblau-Eosinfärbung, *Ibid.* 27: 127, 1901 a.
- Michaelis, L.: Das Methylenblau und seine Zersetzungsprodukte, *Centralbl. f. Bakt., I. Abt.* 29: 763, 1901 b.

stain is a single buffered solution, it was easy to maintain the dye concentrations at a uniform value and change the solvent at will. Thus it became possible to evaluate the rôle of the solvent in the total staining effect.

#### HISTORICAL

The historic aspects of this problem are closely related to the development of knowledge concerning blood stains and an adequate presentation cannot be entered into here. Reference to Conn's (1929) chapter on compound stains will be helpful.

Careful examination of the original publications of the investigators whose stains are in common use today fails to reveal any statement specifically in validating the use of acetone. On the contrary compilation of data from a number of papers dealing with blood stains revealed that the splendid results obtained with acetone solvents had been noted by several workers.

The first investigators to obtain Romanowsky effects mixed separate aqueous solutions of methylene blue and eosin just before use, as Romanowsky (1890, 1891) himself had done. Malachowski (1891) discovered simultaneously with Romanowsky the same valuable staining effects and also learned that replacement of ordinary methylene blue by a polychromed solution yielded satisfactory results consistently. He employed separate aqueous solutions of polychromed methylene blue and eosin, as did Rosin (1898), Nocht (1898, 1899), and Ziemann (1898).

Further investigation showed that the active stain was a compound dye, which was then separated by Reuter (1901) and Leishman (1901) as the insoluble eosinates of the basic dyes present in polychromed methylene blue. The precipitate was dissolved in alcohol, and the solution used as a fixative, water being added just before staining. Reuter recommended absolute ethyl alcohol while Leishman followed Jenner (1899) in employing pure methyl alcohol as the solvent. Leishman laid no special emphasis on the grade of methyl alcohol and later (1904) agreed with Tulloch (1904) that methylated spirit was as satisfactory as pure methyl alcohol. Wright (1902, 1910), who modified Leishman's stain slightly, likewise attached no significance to the purity of the methyl alcohol.

Reuter (1902) was the first one to stress a particular quality of methyl alcohol as desirable. In discussing Leishman's stain, he wrote, p. 843: "Especially valuable for hastening the staining process is the use of that excellent solvent, methyl alcohol (acetone free, purissim, Merck)."

At this early period Michaelis (1901 b), under the influence of Paul Ehrlich and Giemsa, under the direction of Nocht, began to apply to blood stains contributions already made by chemists in the purely chemical studies of methylene blue and its derivatives. Michaelis's stain was essentially like Nocht's. Giemsa's first (1902 a, b) solvent was quite similar, but the stain differed from those of all previous workers in containing the basic dyes in a purified state. These dyes were weighed out accurately instead of being employed in unknown variable amounts in the form of polychromed methylene blue solutions. Two aqueous solutions were mixed, one containing azure II

## AN EVALUATION OF THE TAKATA-ARA REACTION FOR DIAGNOSIS OF LIVER CIRRHOSIS\*

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A YEAR ago we began using the Takata-Ara reaction as described by Heath and King<sup>1</sup> as an additional laboratory procedure in the study of gallbladder and liver disease. After a short experience with the test we were not convinced that it was of much value. This report contains our data on the test as applied to liver disease and many other cases selected at random from a general hospital.

### METHODS

The Takata-Ara test was carried out according to the directions given by Heath and King,<sup>1</sup> using six test tubes. Results were expressed according to their criteria: a strong positive reaction (+++) has complete precipitation in at least one tube or flocculation in five tubes, a positive reaction (++) has almost complete precipitation in one tube or flocculation in three tubes, a weakly positive reaction (+) has a minimum of flocculation in one or two tubes, a questionable reaction (?) has only questionable flocculation, while a negative reaction (-) has no flocculent precipitate in any tube. The test was read sixteen to twenty-four hours after setting it up.

For the majority of the cases, serum was used from whole blood allowed to clot. In some cases oxalated plasma, free from hemolysis, was used.

Protein determinations were done by the Bowman<sup>2</sup> method, ieteric index by colorimeter comparison of serum, suitably diluted with physiologic saline, with a 0.01 per cent solution of  $K_2Cr_2O_7$ .

### RESULTS

We have 21 cases on which a diagnosis of cirrhosis of the liver was made. These are given in Table I. Unfortunately only three cases in this group had the diagnosis confirmed by autopsy. Of these, two gave a positive (++) reaction and one gave a weakly positive (+) reaction. With clinically diagnosed cirrhosis of the liver we have 11 strongly positive (+++) reactions on 8 cases, 6 positive (++) reactions on 6 cases, 9 weakly positive (+) reactions on 4 cases, 4 questionable (?) reactions on 4 cases, and three negative (-) reactions on 2 cases.

According to Crane<sup>3</sup> only the strongly positive (+++) reactions are significant. If so, then our three autopsied cases did not give a positive reaction, and on the clinically diagnosed cases only 8 out of 24 determinations gave a positive test for cirrhosis. Though it is possible that some of the latter group did not have cirrhosis of the liver, the failure of the test on the autopsied cases

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The solvent, when this rapid technic with acetone was employed, consisted of

Glycerin	12.5 parts
CH <sub>3</sub> OH	37.5 parts
Acetone	50.0 parts

Giemsa never developed acetone as a solvent, although Schilling Torgau (1913) diluted Giemsa's stain with an equal part of acetone to obtain an excellent blood and parasite stain, with good keeping qualities. Pappenheim's (1911) "panchrom" stain also contained acetone.

During the World War, when the French supply of Giemsa's stain was exhausted and could not be replaced, a group of French investigators undertook the preparation of their own stains. Among others developed, two are pertinent to the present discussion because of their acetone content. Cara georgiades (1918) used this solvent:

99 per cent CH <sub>3</sub> OH	8 parts
Glycerin (30°)	1 part
Acetone	1 part

Jonesco Mihaiescu's (1918) solvent was as follows:

Glycerin	17 parts
CH <sub>3</sub> OH	74 parts
Acetone	9 parts

Finally, it is interesting to note that Jagic (1906) found acetone to be an excellent fixative for blood smears. Giemsa (1935) also mentioned its use for this purpose.

#### MATERIAL AND METHODS

The dyes and reagents employed, including the buffer solutions, have already been described (Kingsley, 1935). The dyes were certified National Aniline and Chemical Company products, and the reagents were all of the C P grade. An analytical balance weighing to 0.0001 gm was used. Complete solution of the dyes in the solvents described often takes six to twelve hours, but should not be hastened by heating, although several hours in a 35° C incubator is permissible. Caution must be exercised to prevent evaporation, especially of the acetone.

The stains were tried on blood smears made in the usual manner and fixed in methyl alcohol for periods varying from one half minute up to several hours. Some of the stains were also used on fixed bone marrow sections and touch preparations, as well as on frozen sections. The staining technic has already been given (Kingsley, 1935) and consists merely in flooding fixed slides with the stain, and then washing off the stain with a current of distilled water. Tissue sections underwent a differentiating process. Microscopic examination with the oil immersion lens determined the efficacy of the stains in producing Romanowsky effects. To facilitate color differentiations of this nature, it is helpful to have a strong yellowish light, which is better than bluish light or daylight.

reaction (protein on 16 cases), 6.34 gm. per 100 c.c.; questionable reaction (protein on 26 cases), 6.50 gm. per 100 c.c.; negative reaction (protein on 7 cases), 7.6 gm. per 100 c.c.

#### CONCLUSIONS

After a survey of our results on 21 cases of liver cirrhosis, and 151 cases which were diagnosed other than cirrhosis of the liver, on which more than 200 Takata-Ara reactions were determined, we feel that the test is not significant enough to be of value in the clinic as an additional laboratory procedure. Ragins<sup>4</sup> has noted the incidence of positive reactions in eclampsia, carcinoma of the liver, infections and endocrine disturbances. We have found the test, depending as it does on the proteins of serum primarily, is not consistent with any clinical diagnosis. A much more valuable procedure, and one which quantitatively measures the changes of an important constituent of the serum, is the Bowman<sup>2</sup> method of serum protein determination. Where facilities are available this may also be extended to include determinations of the albumin to globulin ratio, in cases where total protein is normal. Myers and Keefer<sup>10</sup> have shown that the A/G ratio is mainly of use in liver disease, as in other disease, to explain peripheral edema.

#### SUMMARY

In our hands the Takata-Ara reaction gave positive results in a large number of cases other than cirrhosis of the liver in which the total protein was low or the albumin to globulin ratio was low.

The reaction was not strongly positive in three cases of liver cirrhosis confirmed by necropsy.

#### REFERENCES

1. Heath, C. W., and King, Elizabeth: The Takata-Ara Test in the Diagnosis of Liver Disease, *New England J. Med.* 211: 1077, 1934.
2. Bowman, R. O.: A Rapid Method for Routine Serum Protein Determination, *J. LAB. & CLIN. MED.* 21: 1092, 1936.
3. Crane, M. P.: A Modified Mercuric Chlorid Reaction (Takata-Ara) in Cirrhosis and in Neoplasms of the Liver, *Am. J. M. Sc.* 187: 705, 1934.
4. Ragins, A. B.: The Value of the Takata and Ara Reaction as a Diagnostic Aid in Cirrhosis of the Liver, *J. LAB. & CLIN. MED.* 20: 902, 1935.
5. Jezler, A.: Die Takatasche Kolloidreaktion in Serum und Körperflüssigkeiten und ihre Beziehungen zu Störungen des Eiweissstoffwechsels der Leber, *Ztschr. f. klin. Med.* 114: 739, 1930.
6. Snell, A. M.: The Effect of Chronic Disease of the Liver on the Composition and Physico-Chemical Properties of Blood: Changes in Serum Protein; Reduction in Oxygen Saturation of the Arterial Blood, *Arch. Int. Med.* 9: 690, 1935.
7. Magath, T. B.: The Takata-Ara Test of Liver Function, *Am. J. Digest. Dis. & Nutr.* 2: 713, 1936.
8. Peters, J. P., and Eisenman, Anna: The Serum Proteins in Disease Not Primarily Affecting the Cardiovascular System or Kidneys, *Am. J. M. Sc.* 186: 808, 1933.
9. Gros, W.: Zur Frage gesetzmässiger Veränderungen des Bluteiweissbildes beim multiplen Myelom. (Zugleich ein Beitrag zur Bedeutung der Bluteiweisskörper für die Takatasche Reaktion im Blut), *Deutsches Arch. f. klin. Med.* 177: 461, 1935.
10. Myers, W. K., and Keefer, C. S.: Relation of Plasma Proteins to Ascites and Edema in Cirrhosis of the Liver, *Arch. Int. Med.* 55: 349, 1935.

The dyes are kept in two separate solutions because the eosinates of the basic dyes precipitate when the solvent contains water. Adjustment of the solvent constituents, however, enables preparation of single staining solutions usable for over a year (see below). The arrangement of the dyes and solvent substances into the two groups shown is based on a large series of experiments which are described in detail in another paper. For the present discussion it is of interest to note that acetone is a better solvent than methyl alcohol for methylene violet, which is an important component of any good Romanowsky stain.

Comparison of stains in which acetone replaces part of the methyl alcohol with those lacking any acetone is given by the two pairs of stains which follow.

Key No	115 -	133	145 -	147
SOLUTION A				
Glycerin	5	10		
*Alcohol	5			
Water	25	30	40	40
Buffer	15	10	10	10
Blue	0.065	0.065	0.030	0.030
Azure	0.010	0.010	0.005	0.005
SOLUTION B				
Violet	0.013	0.013	0.010	0.010
Eosin	0.045	0.045	0.050	0.050
Glycerin	5			
*Alcohol	10	50	15	50
*Acetone	35		35	
Minutes	7	10	4	6
Results	excellent	excellent	excellent	good

It is evident that the acetone containing stains act more rapidly than those without acetone. To obtain equally satisfactory results, the stains lacking acetone require a longer staining time. In No. 147 above, for example, six minutes is not sufficient to obtain a satisfactory stain, but eight minutes yields results equal to those given by No. 145 in four minutes.

Although the above stains all slowly precipitate after mixing, because of their water content, the following formula demonstrates that even after mixing Solutions A and B, the final stain may be kept for at least thirteen months, ready for use by merely flooding fixed slides with it.

SOLUTION A		SOLUTION B	
Glycerin	5 cc	Glycerin	5 cc
CH <sub>3</sub> OH	5 cc	CH <sub>3</sub> OH	10 cc
Water	25 cc	Acetone	35 cc
Buffer pH 6.9	15 cc	Methylene violet	0.010 gm
Methylene blue	0.005 gm	Fuchsin	0.036 gm
Methylene azure A	0.010 gm		

Mix equal volumes of A and B before use.

The best general stain prepared in these experiments has a slightly greater quantity of methylene violet and of eosin. A brief explanation of it has already appeared (Kingsley, 1935).

Finally, acetone was tried as a solvent for certified Wright's blood stain. It was found to be a poor solvent for the powdered stain, resulting in a very dilute solution which must act on slides for about twenty minutes to get satisfactory results. But when 10 to 30 per cent of acetone was added to an

weaker if the reagent is afterward added. It is obvious that the resulting suspension has to be homogeneous to get accurate results. This can only be accomplished by distilling rather small amounts of acetone, so that it is necessary to do a preliminary estimation. Amounts of acetone larger than 0.5 mg. cause such a rapid precipitation that it is impossible to measure turbidity caused by direct distillation accurately. The optimal amount of acetone is about 0.1 mg., which is found to be the average amount in 100 c.c. of normal urine.

A difficulty which has not so far been solved entirely satisfactorily is the *nature and the preparation of the standard solution* used to compare the turbidities.

Folin and Denis are using a standard acetone solution which, although it seems to be most logical, has disadvantages. Since in Folin's procedure the reagent is added to the distilled acetone, much too large amounts of material are required (about 100 to 200 c.c. of blood, 500 c.c. of urine). On the contrary by distilling the acetone into the reagent a strong enough turbidity is produced by the use of 10 c.c. of normal blood (since 0.03 to 0.05 mg. of acetone can still be measured accurately). But in this modification the Folin and Denis standard acetone solution cannot be used, because the reagent has to be added to it afterward. The turbidity obtained is more than about 10 times too weak. It also seems as if this standard solution is not stable on standing and must be restandardized from time to time. It would be better to distill with each test in a second distillation apparatus a known amount of acetone as standard.\* An aqueous mastic solution is quite suitable. The preparation of it is simple. Ten cubic centimeters of a 10 per cent alcoholic mastic solution are run slowly, while shaking, into 500 c.c. of distilled water, 30 c.c. of a 30 per cent solution of gum arabic are added and water up to 100 c.c. It is best to let this solution stand for a few months, to allow impurities to settle out. After centrifugation a stock solution is obtained which is homogeneous and remains constant. By dilution of the stock solution a standard solution may be prepared which is constant for a few days only. The stock solution is the same as the one used in the turbidimetric determination of the proteins in spinal fluids.<sup>5</sup>

The *standardization of this solution* is quite simple. An acetone solution the concentration of which was determined by the Messinger-Huppert method is distilled into the Scott-Wilson reagent and the resulting turbidity compared with the mastic standard. It is preferable to standardize so that the mastic standard equals exactly 0.1 mg. acetone in 100 c.c. By using the mastic standard it is possible to compare a freshly distilled acetone solution with the unknown, without distilling the standard acetone every time.

The error caused by the comparison of a newly distilled acetone solution with an older one is thus eliminated. The *procedure for the acetone determination* is quite simple. One hundred cubic centimeters of normal urine which has to be protein-free are acidified with 1 c.c. of 25 per cent sulphuric acid in the distilling apparatus as described by Scott-Wilson. It is

\*This complication may be avoided by the use of a permanent standard solution fixed once and for all against a known amount of distilled acetone.

- Nicholson, D Laboratory Medicine 1930, Lea & Febiger
- Nocht, Dr Zur Färbung der Malaria Parasiten Centralbl f Bakt, I Abt 24 839, 1898
- Nocht, Dr Zur Färbung der Malaria Parasiten Ibid 25 764 1899
- Pappenheim, A "Panchrom," eine Verbesserung der panoptic Universalanfärbung für Blutpräparate jeder Art nebst Ausführungen über metachromatische Farbstoffe und die metachromatische Potenz des polychromen Methylenblau (Unna), Folia haemat 11 194, 1911
- Reuter, K Ueber den färbenden Bestandteil der Romanowsky Nochtschen Malaria Plasmodienfärbung, seine Reindarstellung und praktische Verwendung, Centralbl f Bakt, I Abt 30 248, 1901
- Reuter, K Weitere Beiträge zur Malaria Plasmodienfärbung mittels A Methylenblau Eosin, Ibid 32 842, 1902
- Romanowsky, D Zur Frage über den Bau der Malaria Parasiten, Wracz, 1890, pp 1171 ff, Russisch Ref by Rothert in Centralbl f Bakt, I Abt 10 163, 1891
- Romanowsky, D Zur Frage der Parasitologie und Therapie der Malaria, St Petersburg med Wehnschr 16 297 and 307, 1891
- Rosin, H Zur Färbung und Histologie der Nervenzellen, Deutsches med Wehnschr 24 615, 1898
- Schilling Torgau, V Note, Folia haemat 15 162, 1913
- Tulloch, F An Alternative Solvent for Leishman's Stain, J Roy Army Med Corps 3 166, 1904
- Wright, J H A Rapid Method for the Differential Staining of Blood Films and Malarial Parasites, J Med Research 7 138, 1902
- Wright, J H Revised Directions for Making and Using the Wright Blood Stain, J A M A 55 1979, 1910
- Ziemann, H Eine methode der Doppelfärbung bei Flagellaten, Pilzen, Spirillen und Bakterien, sowie bei einigen Amöben, Centralbl f Bakt, I Abt 24 945, 1898

urine. Oxybutyric acid in urine as well as acetone and oxybutyric acid in blood, do not show those small deviations so well. It is therefore very important to keep the urine sterile. The commonly used *urine preservatives* are not very efficient. Acidifying the urine with sulphuric acid was found the best, but then only the total acetone can be determined and the separate determination of acetone and acetoacetic acid is impossible. The daily output of acetone in urine was found in the normal to be between 1.2 and 2 mg.

It is advisable to estimate the acetone content of one or more urine fractions on several days to find a very small deviation of the normal. If the amount of the *daily output* is wanted, it is better to determine the freshly voided urine separately than to collect the full twenty-four-hour urine. An increase of the acetone concentration up to 0.2 to 0.3 mg. per cent seems to have more importance than the determination of the total daily output. It is obvious that the increase of acetone after fasting, as it occurs in normal persons, has to be accounted for. A tolerance test may be based on this fact.

#### DISCUSSION

The described method may be designated as a diaphanometric one, according to Mestrezat,<sup>7</sup> who wished to emphasize that the turbidity by means of the naked eye may be compared with a series of standard turbidities. The use of optical instruments may be dispensed with, since the differences of 0.05, 0.1, 0.2, 0.3 mg. in the range of magnitude of 0.03 to 0.3 mg. per cent acetone can be distinguished very sharply with the naked eye. If greater amounts of acetone have to be determined as in the determination of oxybutyric acid according to Schaffer-Marriott or in pathologic increased acetone excretion, the method will not give as good results. The turbidimetric method of Folin or the iodometric method of Messinger-Huppert is preferable here. The urine would have to be diluted so much that the error of dilution would alter the results considerably. A principle which distinguishes the turbidi- and diaphanometric methods from the volumetric or gravimetric method is the fact that the turbidity methods require a certain optimal dilution of the unknown, 0.1 mg. per cent in the one described here and 0.5 mg. per cent in Folin's method. It is an advantage that these methods allow one to determine such small amounts accurately, but in severe pathologic conditions it might limit the use of the method. The turbidity methods are the less accurate when more acetone is present, while with the volumetric or gravimetric methods, the contrary is true and reliable results are obtained if more than 20 mg. per cent acetone are present. The diaphanometric method with the direct distillation seems to be therefore the method of choice for the acetone determinations in blood and urine in the normal range of magnitude; this is a concentration of 0.05 to 0.3 mg. per cent. The iodometric method will totally fail here and the method of Folin would need much more material to start with. But variations of 20 times the lower acetone concentration may occur in this range of magnitude not to be measured by any of the other methods. The method may be applied to urines with a negative Legal acetone test (a concentration below 1.7 mg. per cent acetone). Acetone amounts of 3 to 20 mg. per cent which in urine will give a weak Legal reaction may be determined best by

is significant, and our results are not in accord with those of Heath and King,<sup>1</sup> Ragins,<sup>4</sup> and Crane<sup>3</sup> in this country, and numerous European workers, references to which are given by Crane<sup>3</sup> and Jezler.<sup>5</sup>

Ragins<sup>4</sup> has noted a correlation of the test with icteric index in cirrhosis. A glance at Table I shows that we have obtained strongly positive reactions with icteric indices of 5 to 140, and in Case 7 the reaction remained strongly positive while icteric index fell from 140 to 36. In this group and also in the others to be shown later we can establish no relation to the icteric index of the serum.

Ragins<sup>4</sup> was unable to find a correlation of the test with albumin, globulin, or albumin to globulin ratio, while others<sup>5,7</sup> have noted that the positive

TABLE I  
CASES DIAGNOSED AS CIRRHOSIS OF THE LIVER

CASE	AGE SEX	DIAGNOSIS	SERUM PROTEIN GM/100 CC	TAKATA APA	ICTERIC INDEX	A/G RATIO
7	38 M	Biliary type cirrhosis ? intrahepatic (C)* obstruction	58	+++ +++ +++	140 64 36	
14	43 M	Fever and convulsions ? cirrhosis (C)	70	?		
15	40 M	Ascites, hypertension, atrophic biliary cirrhosis (C)	47 53	++ +++	7 5	
16	48 M	Diabetes mellitus, portal cirrhosis (C)	38 41 64	?	3 6 3	13 19
19	50 M	Portal cirrhosis (C)		+ +	51 34	
36	60 M	Cardiovascular disease, ? portal cir- rhosis (C)	11 63	? +		
40	65 M	Atrophic portal cirrhosis (A)	57	+	14	
64	64 M	Lyonnec cirrhosis esophageal varices (C)	57 44	+++ +++		
67	66 M	? Cholelithiasis, ? biliary type cirrhosis (C)	64 62 61 65 51	+ + + + +	70 36 1 61 62	
72	60 F	Lyonnec cirrhosis (C)	53 42	++ +++	18 9	
98	68 M	Portal cirrhosis (C)	68	+++	8	
101	69 M	Portal cirrhosis (A)	49	++	10	
120	60 M	Portal cirrhosis (C)				
121	59 M	Cardiovascular disease, ? cirrhosis (C)		+	5	
147	68 M	Cardiovascular disease, ? portal cir- rhosis (C)	50 49 61	++ +++ ?	5 6 5	
138	50 M	Duodenal ulcer, portal cirrhosis (C)	66	+ ++	11 6	
159	59 M	Cardiovascular disease portal cirrhosis (A)	40	++	7	
165	64 F	Ascites, obesity, ? cirrhosis (C)	47	++	6	
168	54 M	Portal cirrhosis (C)	65	+++	18	0
171	50 M	Portal cirrhosis (C)		++		
172	50 M	Portal cirrhosis (C)	57	+++	5	

\* (C) clinical diagnosis (A) autopsy diagnosis

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**UNDULANT FEVER**, Diagnosis of, Keller, A. E., Pharris, C., and Gaub, W. H. J. A. M. A. 107: 1369, 1936.

The data presented from a study of 29 cases agree with those reported by Huddleson and his associates for individuals who are either in the active stage of undulant fever or who have recovered from brucella infections and in persons who are living or working under conditions of exposure to this group of organisms.

The results of the intracutaneous and the opsonocytophagic tests in a group of forty-four patients with a wide variety of febrile and nonfebrile conditions indicate that the tests are probably specific for brucella infections. In one patient in this series a diagnosis of undulant fever was made by means of these tests. In all but two patients, who had agglutination titers of 1:20 and 1:40, these tests were negative.

In evaluating the three tests as to their usefulness in the diagnosis of undulant fever, the results obtained indicate that the agglutination test is most dependable in patients who are in the active stage of the disease or who have recently recovered. In individuals who have been infected with brucella the agglutination test may or may not be positive. Under these conditions results obtained from the examination of serums from groups of individuals to determine the incidence of undulant fever would not be reliable. It is also possible that in patients suffering from other diseases a low titer agglutination may be obtained which may be suggestive of infection with brucella but which is not diagnostically significant.

The intracutaneous test is probably the most dependable procedure in determining an allergic state resulting from brucella infection. It was positive in each of the four patients with undulant fever and in twenty-nine, or 5 per cent, of 576 persons living or working under conditions favorable to infection with brucella, while only one of the forty-four patients diagnosed originally as having conditions other than undulant fever showed a positive skin test. The intracutaneous test indicates a state of allergy resulting from infection with brucella and may be used as an epidemiologic procedure to determine the incidence of brucella infection. A positive skin test may indicate infection or may be found in an individual who has been infected but who has developed an immunity to brucella.

To determine the immunity status of individuals, the opsonocytophagic test may be employed in conjunction with the intracutaneous test. The absence of marked phagocytic activity of the polymorphonuclear leucocytes in a patient with a positive skin test indicates infection and a lack of immunity. The presence of marked phagocytic activity would indicate either a developing or an established immunity. If marked phagocytic activity and a positive skin test are demonstrated in a patient with fever, it is likely that the fever is due to some disease other than undulant fever. These tests, therefore, may be used as valuable aids in differential diagnosis.

The results of these observations indicate that the intracutaneous test may be used to determine a state of allergy resulting from brucella infection. This test alone may be useful in determining the presence of infection with brucella in individual patients or the incidence of this infection in groups of the population. However, it gives no indication of the immunity status of the patient. This may be determined by means of the opsonocytophagic test. It is possible with the use of these two tests to determine whether individuals are susceptible, infected or immune with regard to undulant fever.

## ACETONE DETERMINATIONS BY AN ACUTE AND SIMPLE DIAPHANOMETRIC METHOD\*

CARL LANGL, M.D., BERLIN, GERMANY

THE generally used method for the determination of acetone in blood and urine is the iodometric method of Messinger Huppert which gives excellent results, when applied to the determination of pure acetone solutions. In blood and urine this method which may be used when large amounts of acetone, at least 5 mg. per cent, are present, fails, however, if the acetone concentration is as small as the normal content. This is the reason why the normal values for acetone are very unreliable.

The iodometric method is valuable only when the acetone concentration is about 5 or 10 mg. per cent, an amount which is so much higher than the amount normally present, that the result will not be influenced by the unavoidable error of 1 to 2 mg. per cent. For the determination of large amounts of acetone as found in medium or high degrees of acidosis, this method, on account of its simplicity and reliability, will be the one chosen.

Much more specific, although not entirely so, is the acetone reagent of Scott-Wilson,<sup>1</sup> an alkaline solution of mercuric cyanide and silver nitrate, an improvement of the Marsh-Struthers<sup>2</sup> reagent.

Scott-Wilson adds to the urine 25 per cent anhydrous sodium sulphate and 1 per cent sulphuric acid. The steam of the distillation in a second distillation flask is washed with strong alkali to free it of phenols, volatile fatty acids and sulphur compounds. It is then brought directly into the mercury reagent through a condenser. After the liquid in the distillation flask starts to boil, the distillation is continued for ten minutes longer. Twenty cubic centimeters of reagent are used for every milligram of acetone expected. Acetone in small concentration of 0.5 mg. causes a prompt formation of a precipitate. Scott-Wilson collects and washes this precipitate and finally titrates its content with thiocyanate.

The original method of Scott-Wilson in our as well as in Marriott's<sup>3</sup> hands did not give as good results as the author claims. The error seems to be caused by the slight solubility of the precipitate so that substantial losses may occur during washing. This is avoided in the *turbidimetric methods* of Marriott<sup>3</sup> and Folin-Denis,<sup>4</sup> which therefore gave much better results. The *turbidimetric method* has the advantage over the *gravimetric* that precipitate may be used even if it is not absolutely insoluble in the washing solution, with which the precipitate would otherwise be treated.

The fact that the turbidity is greater if the acetone is distilled directly into the Scott-Wilson reagent, than if the reagent is added to the distillate, is of utmost importance for this method. Using the direct distillation, 0.01 mg. in 100 c.c. will give a distinct turbidity, while the turbidity is about 10 times

\*Received for publication, April 16, 1936

**LEPROSY, An Investigation Into the Thick Blood-Drop Method of Diagnosis in, Clouston, T. M. Med. J. Australia 11: 430, 1936.**

The following method was tried in 73 cases:

The blood was taken from the apparently normal thumb or finger. The first drop was wiped away and a thick drop preparation was then made. This was then dried in air, under glass covers, dehemoglobinized in distilled water, and again dried as before. The film was then stained by the Ziehl-Neelsen method, 5 per cent sulphuric acid being used for decolorization. Counterstaining was carried out with aqueous methylene blue for three minutes.

This method is not claimed as original, but it is claimed that it is adequate to demonstrate acid-fast bacilli if any are present, since 14 out of 18 nodular cases investigated gave positive results.

Two films only out of 34 from patients with fairly active cutaneous infections who showed also neural involvement were after prolonged search found to contain but few acid-fast bacilli; none was found positive out of 21 films obtained from patients with inactive or very mild neural infections, ten obtained from suspects and eight from children of lepers.

It is claimed that this method is proved to be of no practical use in the detection of early or latent leprosy.

**VITAMIN D, Estimation of, in Blood Serum, Warkany, J. Am. J. Dis. Child. 52: 832, 1936.**

The following conclusions were drawn from animal experiments:

After oral administration of 0.1 c.c. of viosterol (100,000 U.S.P. units) to rabbits there is an elevation of the vitamin D level within six hours. After twenty-four hours the maximum, generally a level of 1,000 rat units (2,700 U.S.P. units) per hundred cubic centimeters, is reached. This value is maintained for from three to four days. The content then slowly decreases and after from four to six weeks returns to normal.

No influence of ether anesthesia, starvation or fever on a high vitamin D level can be demonstrated.

Elevation of the vitamin D level after administration of 0.1 c.c. of viosterol (100,000 U.S.P. units) occurs without change of the phosphorus, calcium, or phosphatase content of the blood or of the phosphatemic curve. For demonstrating changes of the vitamin D content in the normal animal direct estimation seems more sensitive than the chemical methods.

After oral administration of 1 c.c. of viosterol (1,000,000 U.S.P. units) values as high as 20,000 rat units (54,000 U.S.P. units) per hundred cubic centimeters of blood serum were found.

In forty-eight estimations of the vitamin D content in normal human blood serums, values of from 17 to 50 rat units (from 45.9 to 135 U.S.P. units) per hundred cubic centimeters were found, with an average value of 36.7 rat units (99.09 U.S.P. units).

**LYMPHOGRANULOMA INGUINALE, The Use of Standardized Mouse Brain Antigen for the Performance of, the Frei Test, Grace, A. W., and Suskind, F. H. J. A. M. A. 107: 1359, 1936.**

Lymphogranulomatous mouse brain antigen prepared and standardized according to the method described by the authors possesses none of the disadvantages of human pus antigen for the performance of the Frei test.

Ninety-five specimens of the authors' lymphogranulomatous mouse brain antigen and 41 specimens of commercial lymphogranulomatous mouse brain antigen were employed for a series of 171 tests in 50 individuals who were known to have had lymphogranuloma inguinale.

unnecessary to add 25 per cent sodium sulphate. The water which is distilled during the distillation is replaced at about the same rate by means of a dropping funnel. The receiver, a graduate cylinder, contains 20 c c of Scott-Wilson reagent in which the end of the vertical condenser is immersed. From the onset of the boiling the distillation is continued for ten minutes. The turbid mixture in the receiver, usually about 80 c c, is then filled to the 100 c c mark and compared with the naked eye with a mastic standard in a graduate of the same size and diameter.

A special turbidimeter is unnecessary because the naked eye is well able to distinguish differences. It is obvious that the unknown is not compared with a single standard but with a series representing 0.01, 0.03, 0.05, 0.01, 0.03, 0.05, 0.1, 0.2, 0.3 mg acetone in 100 c c. One is able to guess to intermediate value very accurately. The method will give its *best result with acetone amounts of about 0.05 to 0.1 mg*, such as is found normally in 100 c c of urine, 0.2 mg per cent are quite rarely found, and 0.3 mg per cent are certainly not normal. An increase of acetone up to 0.3 mg per cent or even to 0.5 mg per cent can not be detected by the iodometric method since the unspecificity of this method causes an error of 1,000 per cent or more.

*Blood* is deproteinized according to Folin with tungstic acid, after coagulation has been prevented with fluoride. One hundred cubic centimeters of the filtrate corresponding to 10 c c of blood are used, the procedure being similar to the one with urine. The normal values which for urine are close to 0.1 mg per cent are not as constant in blood; values between 0.1 and 0.5 mg per cent are found. The 0.03 mg per cent acetone which is found in the average in 10 c c of blood can be estimated with the naked eye quite accurately, smaller amounts are less easy to estimate. The *specificity* of the Scott-Wilson reagent is very high, but not an absolute one. Sources of error may occur in the urine, while they are not observed in the blood. An infection of the urine by bacteria will prohibit the determination by either method. High acetone values are found though the Legal test was negative, which certainly are false. Since the Legal test is not very reliable, a control is advised. The urine is acidified and boiled until all the acetone is eliminated. After addition of 2 mg per cent acetone the Legal test is repeated which should now be positive. Urine may contain a substance which inhibits the Legal test even if 6 to 8 mg per cent of acetone are added.

Formic acid, formaldehyde, acetaldehyde are substances which will react with the Scott-Wilson reagent and which cannot be eliminated with security by washing the steam through boiling alkali. Acetaldehyde may be destroyed before the acetone is distilled, although this is unnecessary, because its presence will not influence the clinical interpretation of the test. It is different with formaldehyde which after urotropin medication will cause totally erroneous results. The presence of formaldehyde and also of formic acid may often result in a black reduced precipitation instead of a milky suspension of the Scott-Wilson acetone compound. Urine which causes such erroneous results should be discarded and more importance laid upon the blood determination. However small deviations from the normal, as encountered in urine of sugar free diabetics, are more easily found in the acetone value of the

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Folin's method, especially if a mastic standard instead of an acetone standard is used. The determination of oxybutyric acid according to Shaffer Marriott in normal blood and urine will also give values in this range of magnitude. The iodometric method is the one of choice for the determination of high acetone amounts such as over 20 mg per cent. If this method is applied according to Messinger Huppert the error of about 1 mg per cent caused by the unspecificity of the reaction may be disregarded in urines of such high concentration.

#### SUMMARY

The iodometric acetone determination in blood and urine according to Messinger Huppert is not suitable for very small acetone concentrations.

The normal total acetone content of urine is about 0.1 mg per cent. The daily excretion is about 1.5 to 2 mg. The error caused by the unspecificity of the iodometric method is about 1 mg per cent, a multiple of the normal value.

The mercury reagent of Scott Wilson is much more specific. Unreliable results in urine may be caused by the action of bacteria or by medication. The turbidimetric acetone determination with the Scott Wilson reagent gives better results than the volumetric mercury determination with thiocyanate.

The direct distillation of the acetone into the Scott Wilson reagent causes a turbidity about 10 times stronger than the addition of the reagent to the distilled acetone solution. This procedure is therefore better suited to the determination of the very small amounts of acetone found normally or in a decreased acetone excretion.

A constant standard is described which is not only more reliable than one freshly prepared each time but also simplifies the determination considerably.

In the determination of normal or slightly increased acetone concentrations an optical instrument is unnecessary. The naked eye is quite sufficient for correct reading diaphanometry.

For values of 3 to 20 mg per cent the turbidimetric method of Folin and Denis is the best, while values above 20 mg per cent are determined most accurately by the iodometric method.

#### REFERENCES

1. Scott Wilson H. A Method for Estimating Acetone in Animal Liquids. *J Physiol* 42: 444, 1911.
2. Marsh, James Ernest and Struthers Robert de Jersey Fleming. Condensation of Ketones With Mercury Cyanide. *Trans Chem Soc Lond* 87: 1878, 1909.
3. Marriott Wm McKim. Nephelometric Determination of Minute Quantities of Acetone. *J Biol Chem* 16: 289, 1913.
4. Marriott, Wm McKim. The Blood in Acidosis From the Quantitative Standpoint. *I Biol Chem* 18: 507, 1914.
5. Folin, Otto and Denis W. Turbidity Methods for the Determination of Acetone, Acetoacetic Acid and  $\beta$  Oxybutyric Acid in Urine. *J Biol Chem* 18: 263, 1914.
6. Lange C. Quantitative Eiweissschemie und Kolloidchemie. *Kolloid Ztschr* 68: 69, 1934.
7. Mestrezat, William. Le liquide cephalo rachidien normal et pathologique. valeur clinique de l'examen chimique, p 12, Paris, 1912, A. Maloine, 681 pp.

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This discussion presents the authors' experience up to the present time with these procedures. Further studies are planned in order to confirm the observations represented in the data covered in this paper.

**TINEA INFECTIONS, A Rapid Cultural Method for the Diagnosis of, Blumenthal, F. L., and Snow, J. S. J. A. M. A. 107: 1367, 1936**

A deep hanging drop slide was washed thoroughly, allowed to dry and flamed. Cover slips were kept in an alcohol-ether solution and were dried by being put in the flame. With a sterile loop a small drop of medium was then carefully placed in the center of the upturned cover slip, scrapings or hairs were added, and the cover slip was quickly turned over and put in place on the slide. If only a small amount of medium was used it would remain suspended in the center of the ring and not run around the margin. The cover slip was then sealed in place with melted paraffin applied to the edges with a heated knife blade. The preparation was incubated at room temperature and examined twice a day with the microscope.

The formula for the medium follows:

Crude maltose of Chanut 4 gm., peptone of Chassang 1 gm., and distilled water to make 100 c.c.

Of the forty-eight cases of tinea infection studied, direct microscopic examination demonstrated mycelial filaments or spores in 33 per cent.

Culture of Sabouraud's agar medium gave a positive growth in 64 per cent of the cases in an average time of 6.1 days.

The hanging drop culture method described in this paper showed a positive growth in 72 per cent of the cases in an average time of 1.8 days.

The hanging drop culture was found to be the most useful laboratory aid in the diagnosis of tinea infections, not only because it is dependable but also because it is simple, inexpensive and requires a very short time to demonstrate growth of the organisms.

**RENAL TUBERCULOSIS Greenberger, A. J., and Greenberger, M. E. Quart. Bull. Seaview Hospital 1: 43, 1936**

Of 500 (tuberculous) cases necropsied by the authors evidence of tuberculous infection of the kidney was found in 252, miliary tubercles being found in 228 or 45.6 per cent.

From their experience the authors conclude that: (a) the presence of tubercle bacilli in the urine signifies a lesion in the kidney, (b) that a tuberculous kidney lesion may heal providing it is a nondestructive miliary lesion and does not occur as a terminal hematogenous dissemination from extensive organ tuberculosis elsewhere. True clinical renal tuberculosis does not heal, (c) in bilateral renal tuberculosis, if an early lesion is present in the so-called "good kidney," the more diseased kidney should be removed, (d) the bladder picture is only of diagnostic aid in advanced cases; early renal tuberculosis rarely prevents bladder involvement.

**AGRANULOCYTOSIS The Effect of Amidopyrin Upon the Red, White and Polymorphonuclear Blood Cells of a Series of 100 Patients, Rawls, W. B. Am. J. M. Sc. 192: 175, 1936**

Exclusive of 4 patients who developed agranulocytosis, there was no appreciable change in the white blood cell counts or polymorphonuclear counts of 100 patients who were given amidopyrin daily for prolonged periods.

There was a significant increase in the red blood cells.

Agranulocytosis developed in 1 per cent of 400 patients who were given amidopyrin medication.

It is concluded that amidopyrin does not produce hematologic changes, except in certain isolated cases where there is probably an idiosyncrasy toward the drug.

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VENEREAL DISEASES WITH THE MEDICAL ASPECT OF  
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By

IRVIN S. KOLL, B.S., M.D., F.A.C.S.

Attending Urologist, Michael Reese Hospital, Chicago

434 pages, with 92 illustrations in the text, and 6 color plates.

## From the Preface

In the presentation of this book the author has had but one idea—the presentation of these subjects in a manner to be of practical value to the physician in the general practice of medicine and as an aid to the medical student. A survey of the textbooks on Urology will convince one that too much is taken for granted. The details of differential diagnosis, pathology and minutiae of treatment are lacking, so that the general practitioner is given little aid.

Experienced urologists are not always available for consultation, the library tells little, and the result is that the patient suffers. The author's effort has been, therefore, to supplement this deficiency, in a concise and clear manner. Most of the subject matter represents personal experience and may differ from the opinion of other urologists.

There are abstracts and quotations where opinions are considered of value although differing from the author's.

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The same antigens were also used in 128 persons who never had lymphogranuloma inguinale for a group of 241 control tests. One hundred and eighty three tests were carried out in subjects both with and without lymphogranuloma inguinale with antigens made from normal mouse brain.

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In none of the 424 control tests was a papule produced as large as 7 mm in diameter. The greater number of control reactions (89.6 per cent) showed papules from 1 to 4 mm in diameter.

The difference between positive and control tests was readily recognizable. The use of a control test with normal mouse brain antigen concurrently with lymphogranulomatous mouse brain antigen was advocated.

No change was observed to occur in either the authors' or commercial lymphogranulomatous mouse brain antigen and normal mouse brain antigen on standing for any length of time up to two years after preparation which would make lymphogranulomatous mouse brain antigen unsatisfactory material for the performance of the Frei test.

Consequently it is felt that standardized lymphogranulomatous mouse brain antigen is the most suitable material for the routine performance of the Frei test.

#### TRICHINOSIS The Incidence of in San Francisco McNaught, J. B. and Anderson E. V. J. A. M. A. 107: 1446, 1936

Digestion of 200 human diaphragms obtained at autopsy in San Francisco from individuals ranging from two to eighty seven years of age revealed 48 (24 per cent) infected with *Trichinella spiralis*.

Examination of diaphragms from 25 newborn infants gave negative results.

Living larvae were found in all the positive cases.

The number of larvae was usually small, being less than 20 to each 50 gm. of muscle in 79 per cent of the cases.

None of the clinical records of the positive cases revealed a definite history of trichinosis.

The highest eosinophile count recorded was 4 per cent.

The heart muscle from 15 patients with trichinous diaphragms was negative.

Microscopic examination of stained slides for trichinella is inadequate.

Since there are no practical methods of inspection for trichinous meat, the consumer must assume the responsibility of preventing trichinosis by thoroughly cooking all fresh pork.

#### DIABETES Carotenemia in Hyman W. J. A. M. A. 106: 2030, 1936

The blood serum carotene curves obtained in ten diabetic children after the administration by mouth of carotene in oil were distinctly different from those obtained in twelve nondiabetic, healthy children and demonstrated that the metabolism of carotene is interfered with in diabetes.

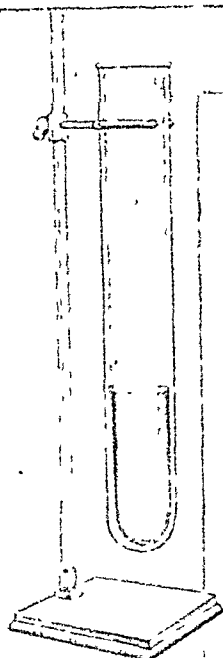
The carotene content of the blood, when it was once increased in the diabetic patients, failed to show the normal decline and remained elevated or even kept on increasing for from ten to fourteen days after the administration of the carotene in oil had been discontinued. The analogy with the hyperglycemic reaction after sugar is given by mouth to diabetic patients is striking and speaks in favor of assuming that the utilization of carotene has been interfered with in diabetes.

The diabetic carotenemia can consequently no longer be explained merely by the high carotene content of the diabetic diet.

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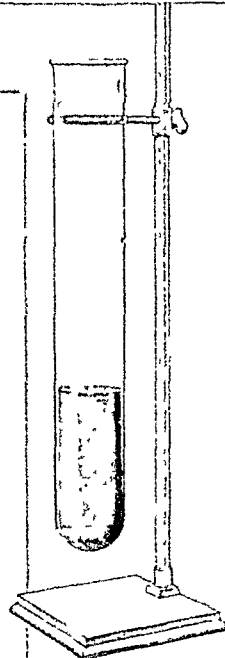
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(Ohio State M. J. 32:123, 1936)

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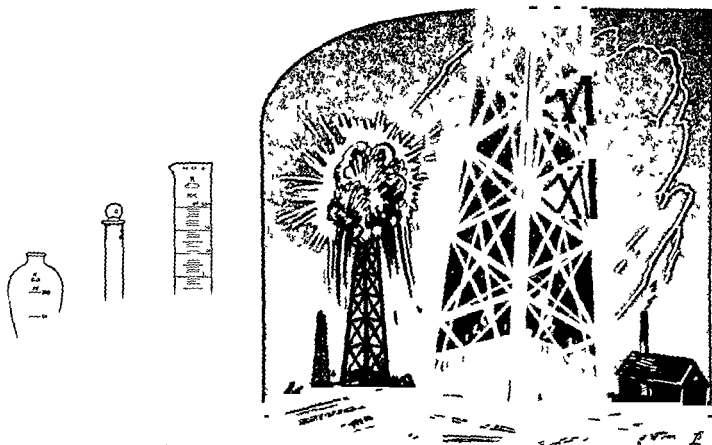
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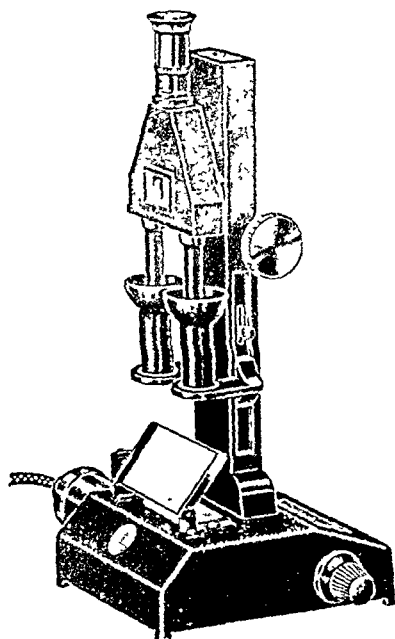
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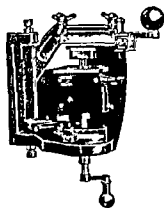
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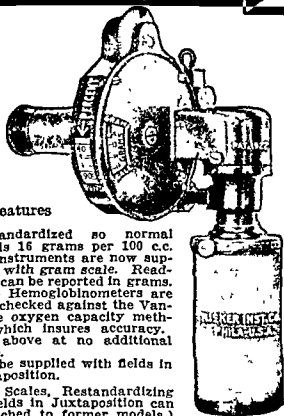
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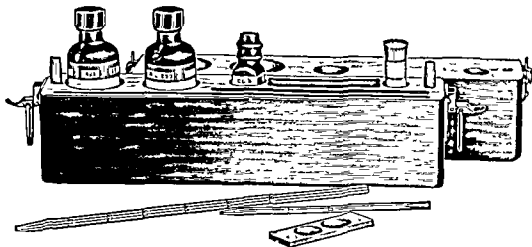
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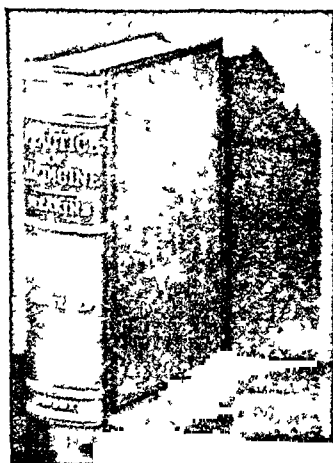
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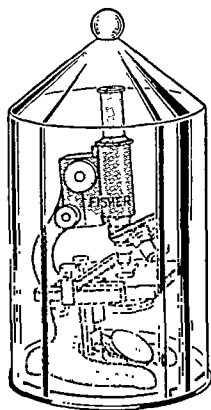
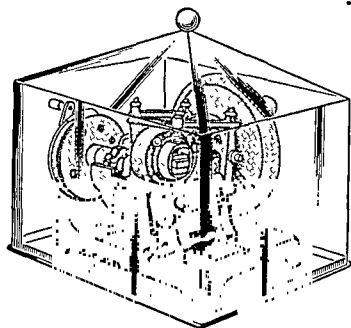
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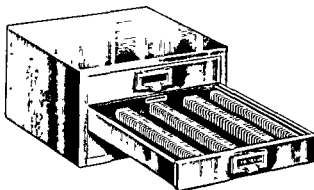
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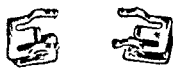
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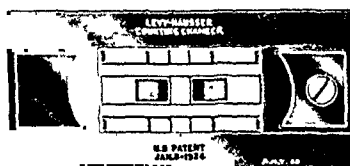
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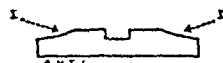


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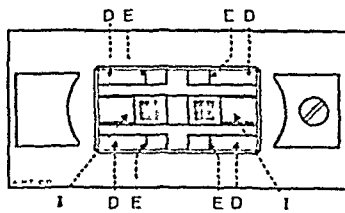


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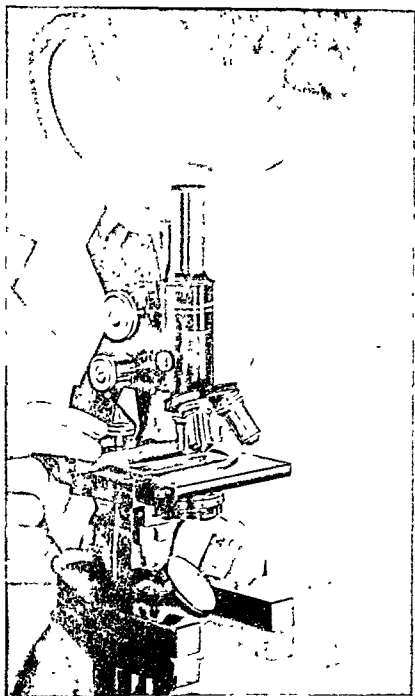
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The association of basophilic aggregation peaks with polar episodes was explained on the basis of irritability of the bone marrow due to spasm of the capillaries and small blood vessels with a resulting anoxia as described in detail in Volume I, Part 2, of *The Patient and the Weather*. A similar explanation has been suggested by Rosegger<sup>2</sup> in a recent publication.

If this explanation is correct, the employment of a pressor active principle should stimulate reticulocyte formation and result in basophilic aggregation peaks higher than those existing as a result of natural occurring meteorologic alterations. With this in view, it was decided to perform experiments with

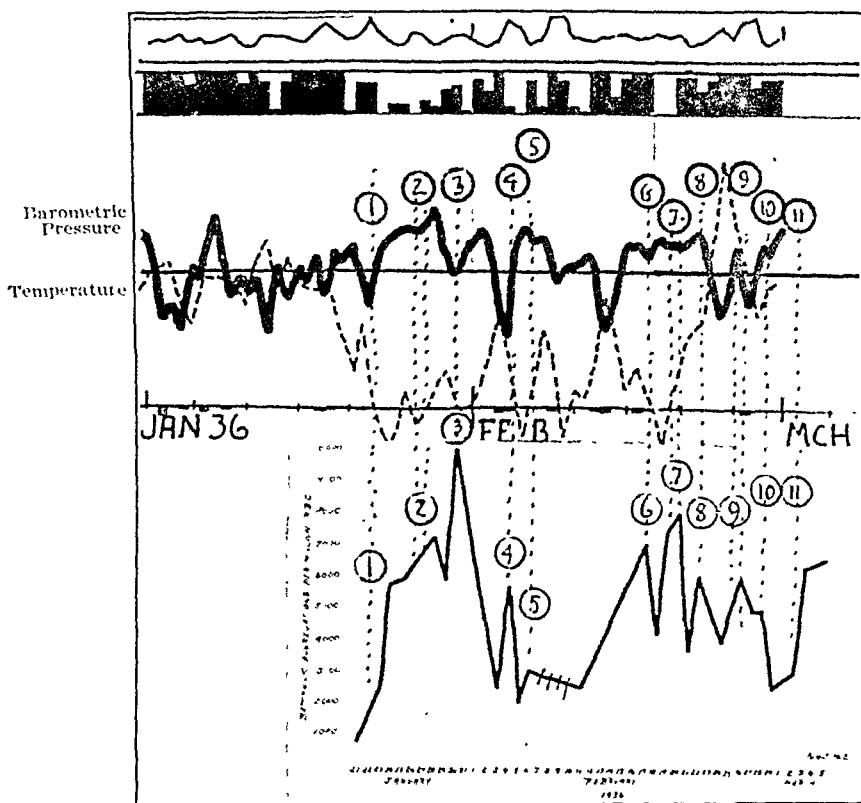


Fig. 1.—Basophilic aggregation counts in Vascular Subject 5 with superimposed meteorograph for the time under consideration. Polar infalls have been numbered 1 to 11 and the corresponding reaction of increase in basophilic aggregates is indicated. In the meteorograph the upper curve is the wind velocity, the black columns the percentage of cloudiness, the heavy line the barograph, and the dotted line the mean daily temperature.

rabbits, employing the pressor principle of the posterior lobe of the pituitary gland. In this manner a controlled method would be at hand whereby, artificially and at any selected time, we could produce a general organic status of spasm and tissue stimulation and irritability (ARS phase)\* which, when the effect was produced in the bone marrow, would be analogous to that resulting with ordinary polar episodes of the cyclonic circulation.

\*ARS phase is the time period during which tissues become stimulated, an acidity develops, metabolism increases, and tissue-stimulating substances are formed because of the relative anoxemia and the interference thereby caused in the course of normal metabolism.



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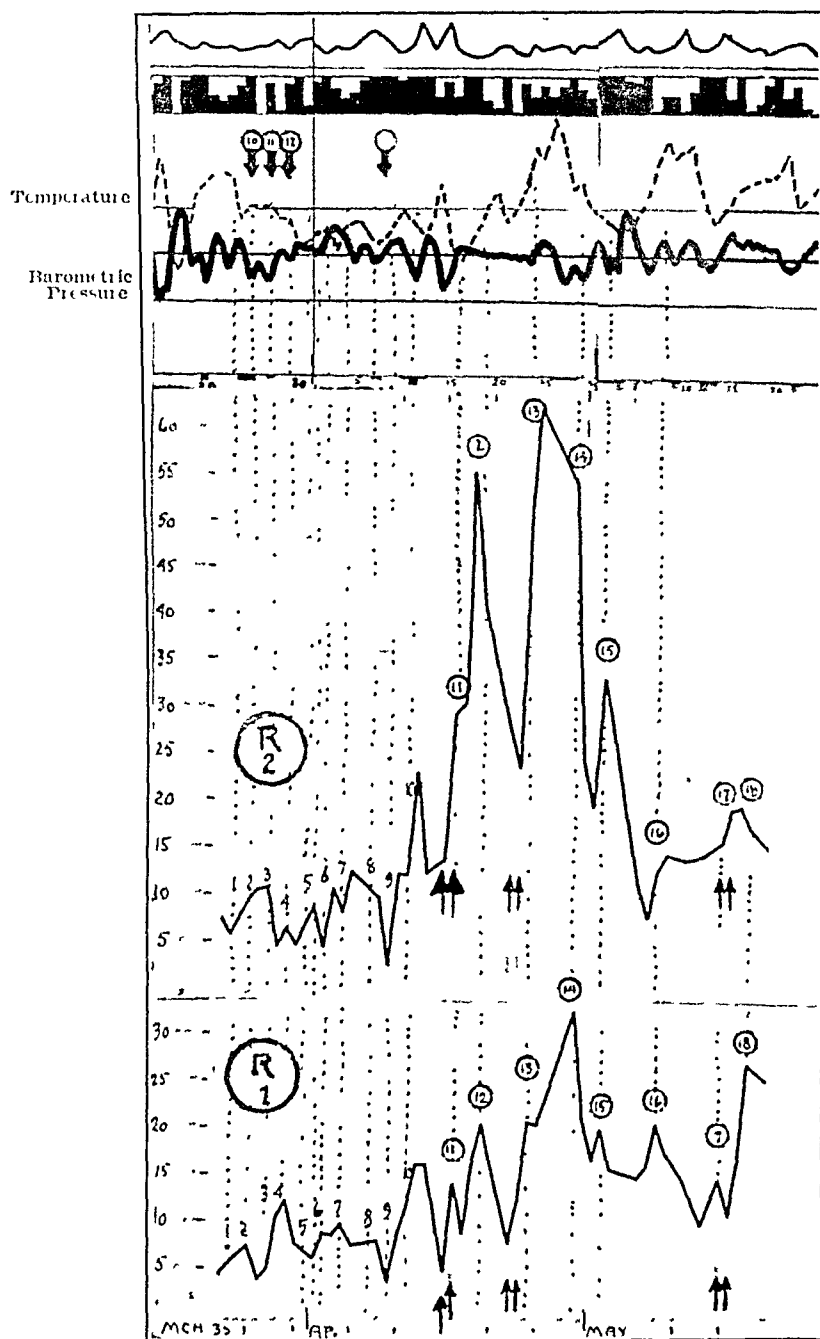
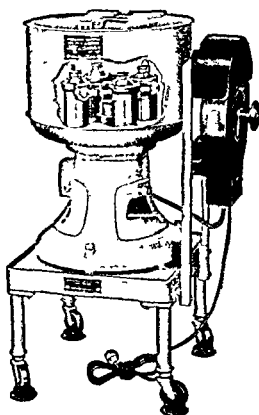


Fig 2—Basophilic aggregation counts in Rabbit 1 and Rabbit 2, with superimposed time under consideration. Polar infalls have been numbered 1 to 18 reaction of increase in basophilic aggregates is indicated. Arrows vs when pitressin was injected (0.5 c.c.).



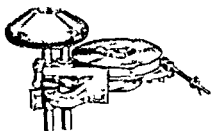
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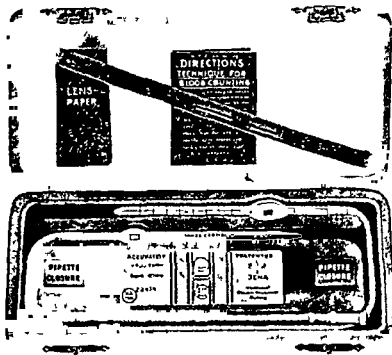
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TABLE II

METEOROLOGIC EPISODES	DATE OF EPISODES	DAYS FOLLOWED BY BASOPHILIC AGGREGATION PEAKS		METEOROLOGIC ASSOCIATION
		RABBIT 1	RABBIT 2	
1 September	10	11		Polar crest
2	12	12	13	Here the polar air masses passed to the north of Chi- cago but without marked barometric alteration
3	15	Crest is extended	16 Extended	Polar infall on eighteenth
4	20	21	21	Polar infall
5	23	23	-	Polar crest
6	25	26		Beginning polar infall
7	26-27	27	27	Polar crest
8 October	1	2-3	2-3	Polar infall
9	3-4	5	5	Polar infall (severe)
10	9	11	10	Beginning polar infall
11	12	14	14	Barometric crest
<i>After Pitressin</i>				
A	21	21	21	Polar infall
B	24	24	23	Polar crest
C	27-28	26-27-28	28	Extension of polar episode
D	31	31	31	Beginning polar infall

## CONCLUSIONS

Analysis of the data obtained, first of all reemphasizes what was observed previously, namely, that in basophilic aggregation counts a normal rhythm occurs, and that the rhythm is meteorologically conditioned.

After the injection of pitressin, the peaks attained were definitely higher than those occurring normally. This is in accord with the assumption that the normal peaks occur as the result of the biologic phase alteration with the anoxia of the ARS phase resulting in bone marrow stimulation. In pitressin we have employed a known pressor agent, and its use has resulted in a summation of natural and artificially produced pressor effects. That a pressor substance will affect the reticulocyte count has been reported by Dodds, Hills, Noble and Williams<sup>3</sup> who found a reticulocytosis as high as 50 per cent five days after the injection of B. P. pituitrin. That anoxia will result in reticulocytosis has been shown by Meyer, Seever, and Beatty<sup>4</sup> who report this finding in rats maintained for twenty hours at a barometric pressure of 282 mm. Our findings confirm the observation of Petersen<sup>5</sup> on the general integration of the environment and the functional status of the organism.

## REFERENCES

1. Gowen, G. Howard: Fluctuations in Basophilic Aggregation Counts With Meteorologic Alterations, *J. LAB. & CLIN. MED.* 21: 677, 1936.
2. Rosegger, H.: Studies on Origin and Clinical Significance of Basophilic Stippling of Erythrocytes, *Klin. Wchnschr.* 15: 158, 1936.
3. Dodds, E. C., Hills, G. M., Noble, R. L., and Williams, P. C.: The Posterior Lobe of the Pituitary Gland and Its Relationship to the Stomach and to the Blood Picture, *Lancet* 1: 1099, 1935.
4. Meyer, Ovid O., Seever, M. H., and Beatty, S. R.: The Effect of Reduced Atmospheric Pressure on the Leukocyte Count, *Am. J. Physiol.* 113: 166, 1935.
5. Petersen, W. F.: Autonomic Integration, The Patient and the Weather 1: Part 2, Ann Arbor, Mich., 1935, Published by Edwards Bros.

# The Journal of Laboratory and Clinical Medicine

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## CLINICAL AND EXPERIMENTAL

### THE EFFECT OF BLOOD PRESSOR EPISODES ON BASOPHILIC AGGREGATION COUNTS\*

G HOWARD GOWEN, M D, SPRINGFIELD, ILL

IN A previous report<sup>1</sup> we have shown that in normal individuals a physiologic rhythm occurs in regard to basophilic aggregation counts as was evidenced by the variations occurring from day to day. In the subjects studied this rhythm coincided with the daily meteorologic alterations, and the observed peaks, in most instances, were in accord with the points of high barometer and low temperature.

This is further exemplified in Fig 1, which is a study made of a hospitalized male† afflicted with arteriosclerosis and possibly periaarteritis nodosa. Examination of the basophilic aggregation curve shows periods of increase which have been numbered 1 to 11 (no determinations February 7, 10, 12, 15) and from which stippled vertical columns have been carried up to the meteorograph. It is noted that episodes 1 to 5 all occur with polar infalls‡. During the period when no counts were made a series of infalls occurred, but after the resumption of the counts we note that episodes 6 to 11 are again all associated with polar infalls. The maximum limits of variation are also noted and are from 750 to 10,000 basophilic aggregations per million red blood cells.

\*From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

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†The patient is described in detail by Petersen as Vascular Subject 5 in Volume IV, *The Patient and the Weather*.

‡Polar Infall is the meteorologic designation for that period in which a mass of polar air is spreading over the earth's surface and is reflected in a rising barometer and a lowering of temperature.

Tropical Front indicates the sudden change from polar air to the typical warm moist air mass which revolves in a counterclockwise fashion and which in America commonly travels from West to East. It is this rapidly moving air mass which characterizes the cyclonic circulation of the northern hemisphere and has its reflection in a low barometer and a high temperature.

A cervical incision was made about 3 inches long. The neck muscles were separated in the midline and retracted laterally. The larynx was exposed and the thyroid gland visualized. The thyroid gland was removed complete after ligation of the blood supply at the upper and lower poles. Attempts in all cases were made to preserve the parathyroid glands in situ. The thyroid glands were placed in moist (Ringer's) warm laparotomy pads and were exchanged with the operator of the second dog. A midline abdominal incision was then quickly made. The peritoneum was opened and the omentum exposed. The capsule of the thyroid gland was fixed to the omentum by three black silk sutures. The omentum was then folded over to form a culdesac containing the gland. The pocket of folded-over omentum was closed by two or three sutures. The lower border of the omentum was always selected, and



Fig 1—Showing thyroid gland in omentum cross planted eighteen days. The alveoli are distended with colloid which is deeply stained. There is no infection or involvement of the omentum

its rich blood supply thus contacted all surfaces of the thyroid gland. Care was taken against strangulating any of the blood vessels by placing the sutures in avascular areas. A special abdominal incision was made and a pocket was formed between the fascia and rectus (9 cases) or between the rectus sheath and peritoneum (9 cases). The pocket was away from the incision and the second lobe of the thyroid gland was placed therein. Closure was made with black silk.

There were no immediate postoperative deaths. Four animals developed tetany of which three improved after treatment with parathormone and calcium chloride. All the animals received desiccated thyroid in their food the first postoperative week. Three animals developed superficial neck infections which cleared with surgical treatment. Only one animal developed an infec-

## EXPERIMENTS

Rabbits were chosen as the experimental animals and the two selected were observed first during the months of March, April, and May of 1935, and then during September and October of the same year. Two contrasting times of the year were selected in order to make note of seasonal variations in addition to daily variations. Pitressin (P. D. & Co.) was employed as the pressor agent. In each instance daily basophilic aggregation counts were made for several days before the utilization of the pitressin in order to determine normal daily variation. The McCord method of staining and counting was employed as in our first report. The pitressin was given intravenously in the marginal vein of the ear at the indicated intervals, and the dosage at each time of injection never exceeded 0.5 cc.

In Fig. 2 are seen the observations made on Rabbits R<sup>1</sup> and R<sup>2</sup> during March, April, and May, 1935. The meteorograph of this period has been superimposed on the charts illustrating the reticulocyte counts. Vertical stippled lines have been carried through the graph on days that are followed by increases in reticulocytes. For the period preceding the injection of pitressin the crests of the curve are numbered 1 to 10, and for the period following pitressin 11 to 18. The synchronization is noted in Table I.

TABLE I

METEOROLOGIC EPISODES	DATE OF EPISODES	DATE OF BASOPHILIC AGGREGA- TION PEAKS		METEOROLOGIC ASSOCIATION
		RABBIT 1	RABBIT 2	
1	March	23		Beginning polar infall
2		25	27	Polar infall
3		27		Polar infall
4		29	29	Polar infall
5	April	1	1	Polar infall, irregular
6		2		
7		4	5	Polar infall
8		7	7	Polar infall
9		9	10	Barometric peak
10		11	12	Beginning polar infall
<i>After Pitressin</i>				
11		15	15	Polar infall
12		20	19	(Probably a polar effect with no barometric change?)
13		24	24	Polar infall
14		29	29	Polar infall
15	May	2	2	Polar infall
16		8	9	Polar infall
17		15	16	Polar episode
18		19	18	Polar episode

It can be seen that the polar episodes on March 23, 25, 27, and 29 find their reflection in reticulocyte increase but with somewhat different summation in the two rabbits. It is also of interest to note that an unusually low count occurred around the ninth of April. With *pitressin injections* the total number of reticulocytes is greatly increased, though here, too, the rhythm of an association with meteorologic episodes is maintained for periods that are not involved in the pitressin injections as with crests 12, 14, 15, and 16.

In Fig. 3 we have the observations made on the same rabbits (1) and (2) during September and October of 1935. For the period preceding the injection



Fig 3—Section of thyroid gland after twenty-eight days implantation in omentum showing round cell infiltration of gland. Acini irregular and faintly staining

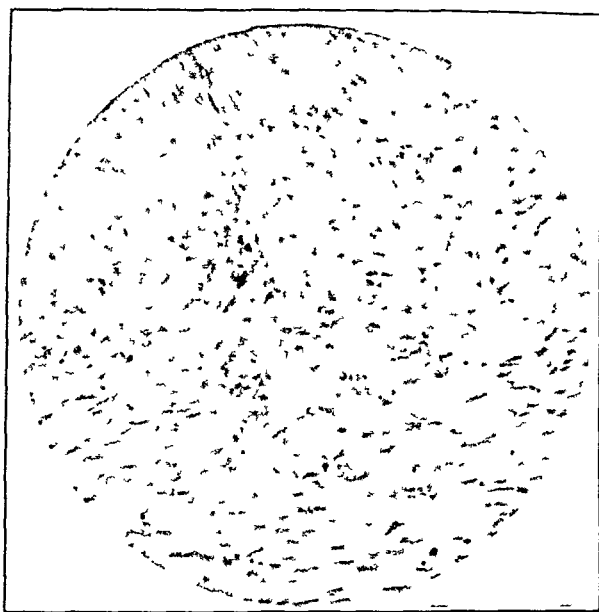


Fig 4—Tissue sectioned through area previously containing thyroid gland. There are practically no evidences of such implantation. Note remnant of black silk at the upper angle

tion of pitressin the days followed by an increase in reticulocyte count, are numbered 1 to 11, and for the period after pitressin, the actual days with reticulocyte crests are designated A, B, C, and D

The synchronization is shown in Table II

Here again we find that the reticulocyte peaks after the injection of pitressin exceed those occurring normally. These peaks are not as high as those pro-

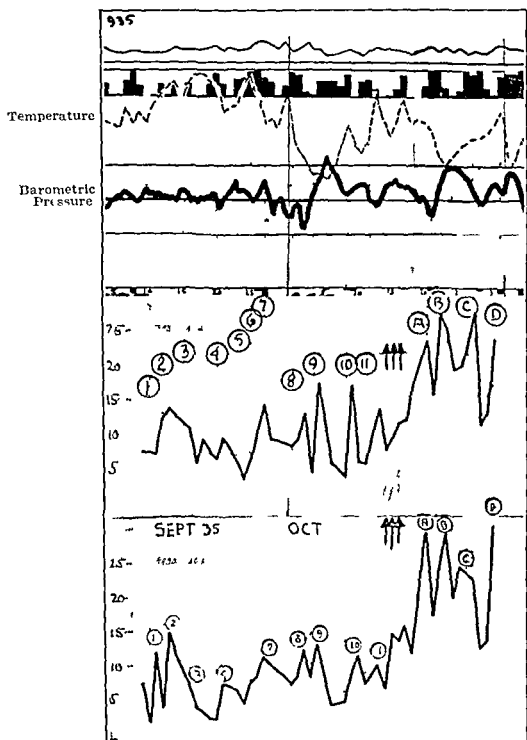


Fig. 3.—Basophilic aggregation counts in Rabbit 1 and Rabbit 2 with superimposed meteorological graph for the time under consideration

duced in the experimentation carried on during March and April, but this would be expected inasmuch as March and April are more disturbed meteorologically than September and October, and the summation of the natural and artificial pressor effects would be less during the less disturbed season. The normal rhythm is maintained in peaks A, B, C, and D inasmuch as each coincides with a naturally occurring polar episode.

animal. In 2 animals, 6 were found, 3 animals showed 5, 2 showed 3, and 12 showed 2. In 4 animals a single glandule was found." This accounts for the positive basal metabolism graphs we obtained. Though this accessory thyroid tissue appears minute in amount, it can sufficiently supply the body demands. We found no hypertrophy of these accessory glandules. Halsted emphasized this fact before.

#### SUMMARY

Thirty-six cross transplants were made in animals whose blood cross matched. After ninety days no evidences of living thyroid tissue could be found. Basal metabolic studies were unsuitable criteria of successful takes, as proved finally on autopsy examination. The existence of accessory thyroid tissue no matter how grossly small was sufficient in dogs to fill body requirements without addition of foreign gland grafts.

The authors gratefully acknowledge their indebtedness to Professor Arthur M. Wright and Dr. CoTui.

#### REFERENCES

- Halsted, W. S.: Surgical Papers vol. II, The Lord Baltimore Press.  
Payr, E.: Thyroid Gland Transplantation, *Arch. f. klin. Chir.* 106: 16, 1914.  
Hesselberg, C.: A Comparison of Autoplastic and Homeoplastic Transplantation of Thyroid Tissue in the Guinea Pig, *J. Exper. Med.* 21: 164, 1915.  
Hess, J. H., and Strauss, A. A.: Autotransplantation and Homotransplantation, *Arch. Int. Med.* 19: 519, 1917.  
Swarts, J. L., and Thompson, R. L.: Accessory Tissue Within the Pericardium of the Dog, *J. Med. Research* 24: 299, 1911.  
Manley, O. T., and Marine, D.: The Transplantation of Ductless Glands, *J. A. M. A.* 67: 260, 1916.  
Holman, E.: Protein Sensitization in Isoskin Grafting, *Surg. Gynec. Obst.* 38: 100, 1924.

## ISOTRANSPLANTATION OF THYROID GLANDS IN DOGS\*

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NEW YORK, N Y

WE HAVE been interested in finding a method of successfully performing glandular homotransplants. The following report constitutes a study of thirty six thyroid cross transplants in eighteen dogs. This study involved cross matching of the blood of dogs, basal metabolisms, postoperative clinical observation, and finally autopsy examination.

The experimental work of Halsted on auto and isotransplantation of the thyroid and parathyroid glands is the greatest individual contribution on this subject. The successful transplantation by Pavy in 1906 of a parathyroid glandule into the thyroid stimulated Halsted to investigate this subject completely. As a result of these studies he formulated the Law of Deficiency. This states that there must be more than a 50 per cent reduction in physiologic activity of a gland before the possibilities of a transplant can be assured. The only autotransplants that took were those in which a deficiency greater than one half was created. Every isotransplant attempted by Halsted in spite of a created deficiency failed. The transplanted material was found completely absorbed or existing as a necrotic mass depending upon the time of inspection after the cross transplantation.

The mechanical factors in transplantation such as asepsis, the site of inoculation whether it be spleen, rectus sheath, or axilla, fragmentation of the gland, et cetera, have in no way contributed to the permanence of homo transplanted glandular tissue. Blood compatibility as a factor has been considered by previous workers, namely Laver and Morris and Hess and Straus. The latter cross transfused their animals before homotransplantation of the thyroids was done. These transplants were all absorbed. Others (Underwood and Holman) believe that foreign protein sensitization is the important factor in absorption.

The work listed below represents eighteen total thyroidectomies and thirty six single cross plants. The average weight of the dogs was about 13 kilos (28 6 pounds). The dogs were operated upon in pairs. The blood of each pair was cross matched and did not agglutinate. A basal metabolism was done in each case prior to operation. On the day of operation the animal received one gram of morphine sulphate followed in a half hour by an intra tracheal anesthesia of ether. Operating room technic and asepsis were rigidly observed in all cases.

\*From the Department of Experimental Surgery, New York University Medical School.  
Received for publication February 20 1936

lished that certain infections and reinfections, and foci of infection, tend to sensitize certain, if not all, individuals to the bacteria and their products of metabolism, to which products these individuals become more or less allergic, (bacterial hypersensitiveness). This allergic manifestation which is present in chronic infection can be readily shown by intradermal testing with the products or soluble toxins of these bacteria. Active clinical disease in a chronic form will occur in such allergic individuals, and a rheumatoid arthritis will develop in a patient who has such a hereditary tendency and constitutional predisposition (arthrotropic). It may also be said that chronic streptococcic disease is a definite clinical entity, as much as chronic tuberculous disease, and the allergic mechanism is probably similar.

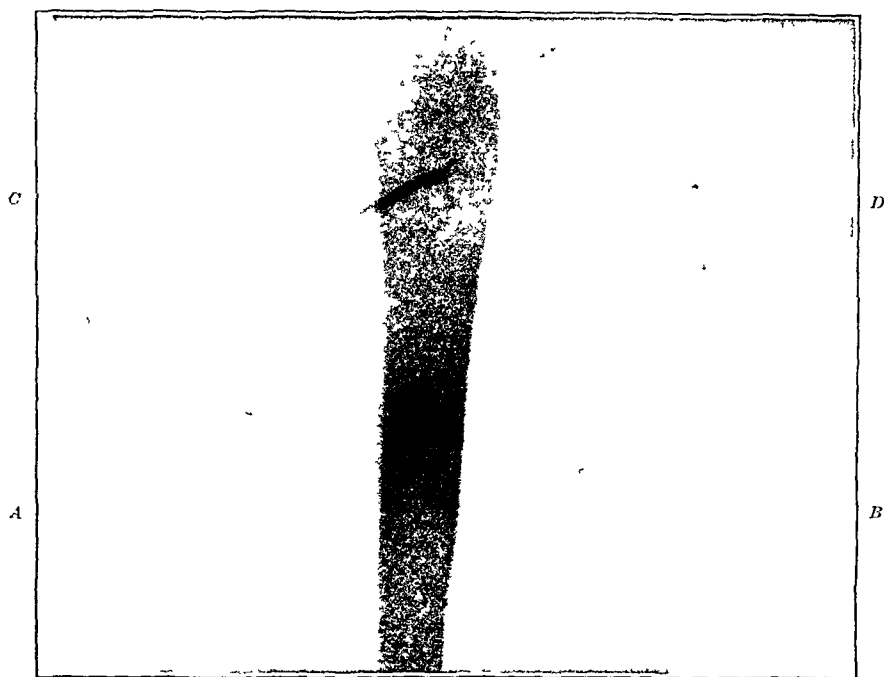


FIG. 1.—Intradermal reactions. A, Streptococcus toxin, immediate reaction. B, Sterile broth control, immediate reaction. C, Streptococcus toxin, twenty-four-hour reaction. D, Sterile broth control, twenty-four-hour reaction. Note that the immediate reactions are alike in both the toxin and sterile broth control.

It was thought, therefore, that if these patients could be treated with the same substances to which they are allergic, beginning with a small dose and gradually raising the dosage in accordance with the patient's reaction or response, there might result an antigenic stimulation of some value. However, the danger of injecting toxin broth filtrate was carefully considered, as it might throw an additional hazard upon subvitalized tissues. It was, therefore, reasoned that if these toxins were detoxified or modified, it might prove an agent of low toxicity and high antigenic value to stimulate a response in these depreciated tissues. This was attempted as follows: Strains of streptococci were isolated from patients with active rheumatoid arthritis. Cultures were

tion of the abdominal pocket. This thyroid was destroyed. Three animals died within two weeks of operation possibly due to acute thyroid deprivation. The first month after operation the animals were thin and lost much of their hair. They improved quickly, however, gaining in strength, weight, and hair growth.

Below we give some brief notes on the autopsy findings of dogs dead on the second postoperative day (tetany), the eleventh day (sacrificed), and the typical findings of those autopsied after three months

Dog 24—The neck incision was opened. A small amount of fluid showed in a clean operative field. The abdomen was opened through the operative incision. The omentum was found to be thickened at its distal portion. The vessels of the omentum were markedly



Fig. 2—Thyroid removed after twenty-one days' implantation. Note the alveoli are distended. The colloid is practically gone with empty acini. There is a moderate amount of round cell infiltration.

injected. The thyroid appeared swollen and injected. The second thyroid under the fascia was hemorrhagic on its upper and lower surfaces. There was no sign grossly of peripheral or central necrosis in either gland.

Dog 4—Eleventh postoperative day. Both thyroid glands were living. The thyroid gland in the omentum was surrounded by blood vessels. The omentum was thin and showed no signs of inflammation. There was no secondary infection or hematoma. The thyroid embedded within the posterior sheath had a definitely established blood supply.

Dogs 66, 33, 26, 22, 35, 62 when sacrificed after three months showed no traces of either the superficial or deep grafts. A deep brownish yellow stain of the tissue and remnants of black silk were the only indications of any previous surgery. The omentum appeared perfectly normal.

The basal metabolic graphs in our series were interesting. The animals showed no lowering of the basal metabolism. Early in this work we considered

with the stock strain giving the strongest reaction. Treatment was given twice a week and continued for at least three to six months, and in many cases much longer. Occasionally a patient reached 0.1 c.c. of a 1:10 dilution and could take no higher dosage, except at the risk of a marked local reaction lasting many days, and at times a focal or constitutional reaction. The latter reactions are uncomfortable, *never dangerous*, but are to be avoided, as they tend to bring the patient into a higher allergic state and interfere with the attempted desensitization.

At first treatment was begun very guardedly in patients with advanced stages of the disease, of many years' duration, and with marked deformities. The clinical results, subjective and objective, were not only encouraging but astounding, even though not manifest at times, before some months had elapsed.

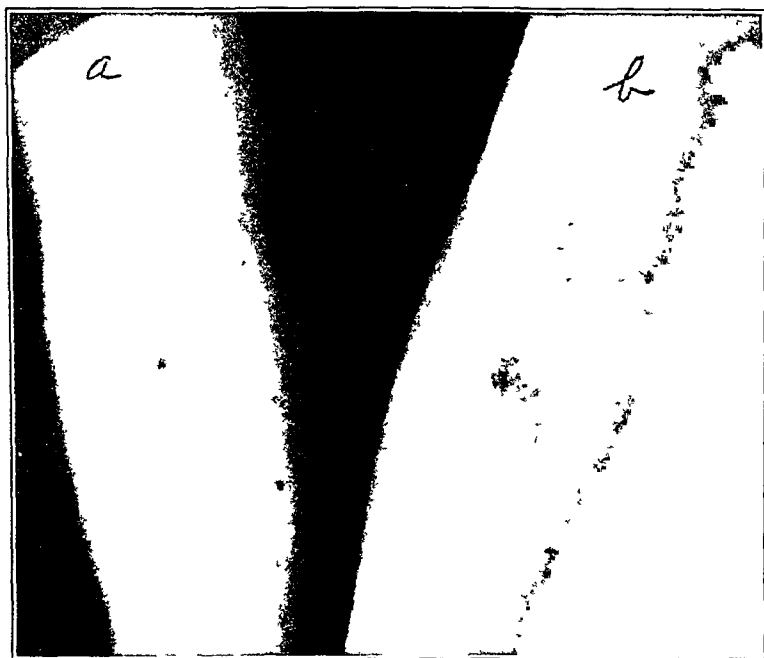


Fig 3—Intradermal reactions A, Tuberculin reaction (Mantoux) forty-eight hours. B, Streptococcus toxin reaction twenty-four hours.

There were objective signs of definitive improvement, locally and constitutionally. Subsequently, treatment was begun on a larger scale, and included many types of patients in various stages of the disease.

#### SOME ILLUSTRATIVE CASES

CASE 1.—R Q., white, female, twenty three years old, bookkeeper, was admitted to the Medical Service of the Kings County Hospital, on Oct. 27, 1932, with the diagnosis of infectious polyarthritis. About six to eight weeks prior to the onset of the arthritis the patient had had a severe grip and had been confined to bed for two weeks. The arthritis was acute, and involved all the joints of the right upper and left lower extremities. The temperature ranged from 101° to 103° F. for two months, and the patient was going downhill rapidly with marked emaciation and excruciating pains in the joints, requiring narcotics frequently. The heart findings were interpreted as due to rheumatic fever, while the changes in the joints as

this as evidence of successful cross transplantation. This view we were forced to discard when the grafts were not demonstrable.

Autopsy examination revealed the presence of small bodies of accessory thyroid tissue located along the arch of the aorta or in the mediastinum of the dog. Halsted first recognized the existence of such accessory tissue but

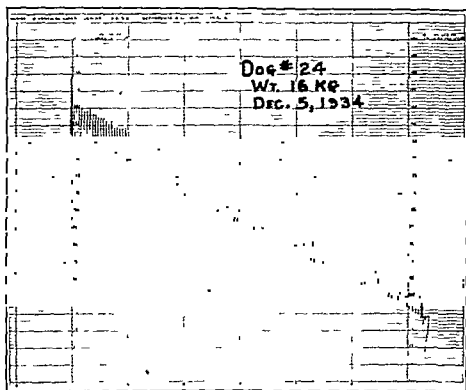


Fig. 5.

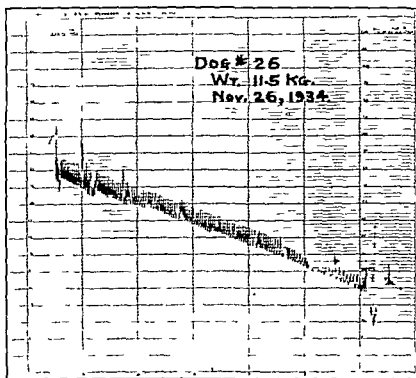


Fig. 6.

Figs 5 and 6—Basal Metabolic Graphs. Dog 26, three months after thyroid transplants. Dog 24, tenth postoperative day.

did not emphasize its importance. Swarts and Thompson found accessory tissue (thyroid) in 24 out of 30 dogs examined. We quote their findings: "Twenty-four of the animals had pericardial thyroid glandules. The total number of the glandules found was 68, as many as 7 being found in a single

per cent polymorphonuclears. The temperature ranged between 98.6° F. and 100.5° F., and the pulse was moderately elevated. Treatment with modified streptococcic toxin was begun in June, 1933, and continued for ten months on the Orthopedic Service of Dr. J. L'Episcopo, which service gave every conceivable help in the rehabilitation of the child. A note made by the Orthopedic Service on the chart on Dec. 12, 1933, states: "knees and ankles freely movable, no pain anywhere, right elbow fixed." The patient was discharged May 2, 1934. She had gained considerable weight, was pain free, and was able to walk without help. This patient was last seen on Oct. 10, 1935. She looks well and feels well, has had no recurrence of pain or any other joint symptoms during the past eighteen months, without any therapy. If it were not for the ankylosis of the right elbow with which she was first admitted to the hospital, it would be difficult to tell that the child had been so severely ill with a deformative arthritis.

CASE 4.—M. M., white, female, fifty years of age, houseworker, was admitted to the Medical Service of the Kings County Hospital on Aug. 9, 1932, with the complaint of pain and swelling of all the joints of the upper and lower extremities of several weeks' duration. She ran a slight fever of 99° to 101° F. for a month. She had had a similar attack three years previously and was bedridden for eight weeks. The present attack was much more severe and the patient continued to get worse with marked emaciation and beginning deformities of hands, wrists, elbows, knees, and ankles, and periarticular muscular atrophy. She was considered a permanent invalid. Consequently she was depressed mentally. A urine examination was negative. Her blood pressure was low, 100 systolic and 65 diastolic. Blood Wassermann was negative and a blood study showed a moderate anemia. Treatment with modified streptococcic toxin was begun in December, 1932, four months after her admission to the hospital, and continued twice weekly for a month and then once a week for four months. She was discharged from the hospital on July 27, 1933, in excellent condition, with perfect use of all her joints, freedom from pain, and with considerable gain in weight. After a rest in a convalescent home for several weeks, she went back to her job, doing housework.

TABLE I

## SUMMARY OF TREATMENT OF 100 CASES OF RHEUMATOID ARTHRITIS

Number of cases	100	
Males	25	
Females	75	
Marked improvement	43	} 69 (favorable results)
Moderate improvement	26	
Slight improvement	16	} 31 (failures)
No improvement	15	
Males	25	
Marked improvement	14	56%
Moderate improvement	6	24%
Slight improvement	3	12%
No improvement	2	8%
		} 80%
		} 20% (failures)
Females	75	
Marked improvement	29	39%
Moderate improvement	20	27%
Slight improvement	13	17%
No improvement	13	17%
		} 66%
		} 34% (failures)
Marked Improvement: Disappearance of pain, swelling greatly reduced or disappeared, function greatly increased or returned to normal.		
Moderate Improvement: Pain greatly diminished or disappeared, but recurs occasionally. Swelling receded, but not completely and permanently. Function improved. Occasional transient exacerbation.		
Slight Improvement: Pain and swelling occasionally disappeared but recurs frequently, especially in change of weather.		

# THE TREATMENT OF RHEUMATOID ARTHRITIS WITH FORMALIZED STREPTOCOCCUS FILTRATE (TOXOID)\*

ABRAHAM S. GORDON, M.D., BROOKLYN, N. Y.

THE subject of arthritis needs no introduction, as the literature on this subject has been abundant during the past decade, due to the interest which has been stimulated in the study of arthritis by the International League for the Study and Control of Rheumatic Diseases.

It is not the purpose of this paper to review the literature of the subject. A complete and detailed résumé of the American and English literature on "Rheumatism" and arthritis was recently published by Hench, Bauer, Fletcher, Ghrist, Hall and White,<sup>1</sup> and the reader is referred to this review for classification, etiology, pathology, laboratory procedures, theoretical considerations and various methods of therapy, as well as the names of all the workers in this field and their contributions.

## TREATMENT

The difficulties in the treatment of rheumatoid arthritis are generally admitted not only because of the different degrees of involvement and stages of the disease in different patients, but also because of the variety of treatments and remedies offered and used, among which are included (1) Many drugs, chemicals and various compounds, including endocrine preparations, (2) diet, (3) vitamins, (4) climate, (5) physiotherapy, including ozone, hot air conditioned cabinets, and colonic irrigations, (6) artificial fever, (7) surgery (synovectomy, arthroplasty, arthrodesis and sympathectomy). Injections used include (8) Many varieties of arsenicals, (9) gold salts, (10) silver salts, (11) iodized oil, (12) colloidal sulphur, (13) histamine, (14) choline salts (The latter two with or without the galvanic current) (15) Many varieties of milk preparations, (16) foreign proteins of all types, (17) typhoid vaccine, (18) other vaccines, in single or multiple forms.

## VACCINE THERAPY

Vaccine treatment with the streptococcus, which has been stressed by many ardent workers and has occupied much space in the literature of recent years, has at best shown but limited success. In general, cases in earlier stages improved much more frequently than those in later stages, in the latter group vaccine treatment did not prove very encouraging.

It was because of the limited success and the unconvincing rationale of therapy that it was decided to broach the subject from a different angle. According to the investigations of Rich<sup>2</sup> and others it has been fairly well estab-

\*From the Arthritis Clinic, The Jewish Hospital.

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TABLE II  
AGE INCIDENCE AND RESULTS THEREIN

AGE IN DECADES	MARKED IMPROVEMENT	MODERATE IMPROVEMENT	SLIGHT IMPROVEMENT	NO IMPROVEMENT	TOTAL PER DECADE
10		1			1
20	3	1			4
30	5	5		1	11
40	16	7	6	4	33
50	10	5	4	4	23
60	8	5	5	5	23
70	1	2	1		4
80				1	1
Total in each group	43	26	16	15	100

## COMMENT

An analysis of Table II shows the age incidence in decades, represented by the last vertical column, and the results of treatment in each decade, shown by each horizontal column, separated into the respective groups. For instance, in the fifth decade, the total number treated is 23. This number is found in the last vertical column, on the horizontal column between 40 and 50 (see Table II). Of these 23 patients, 10 were markedly improved, 5 moderately improved, 4 slightly improved, and in 4 there was no improvement at all. The same analysis is made of the groups in other decades. The bottom horizontal column gives the total number in each group of results, including all ages. The last number in this column gives the total of all these groups as well as the total of all decades in the last vertical column.

## SUMMARY

An analysis of 100 cases of rheumatoid arthritis is herewith presented. Treatment with formalized bacterial filtrates was based on the premise that chronic nonspecific infectious arthritis presents a problem of reinfection of a previously sensitized host akin to the mechanism now accepted in tuberculosis. The results justify this method of approach and warrant its continuation. Whether the procedure involved represents the usual nonspecific protein treatment or the so-called anamnestic reaction is difficult to tell at present. Continued observations and studies of these patients from different angles as to the effect of treatment and the body responses caused by this effect may throw further light on the value of this therapy.

I wish to acknowledge my indebtedness to Dr. B. Koven, who is responsible for stimulating the initiation of this study and who was generous in the supply of material from his Orthopedic Service at the Jewish Hospital. Also, I wish to express my gratitude to Dr. J. L'Episcopo, Chief Orthopedic Surgeon of the Kings County Hospital, who was keenly interested in the problem and who permitted the use of the material in his orthopedic wards for this study.

## REFERENCES

1. Hench, P. S., Bauer, W., Fletcher, A. A., Ghrist, D., Hall, F., and White, P.: The Present Status of the Problem of "Rheumatism"; A Review of Recent American and English Literature on "Rheumatism" and Arthritis, *Ann. Int. Med.* 8: 1315, 1495, 1673, 1935; also 9: 883, 1936; and 10: 754, 1936.
2. Rich, A. R.: Nature and Rôle of Bacterial Allergy, *Lancet* 2: 521, 1933.

made from roots of teeth, tonsils, throat, nasopharynx, nose, sinus puncture, urine, and stool. These were grown in beef heart broth, with a pH of 7.7 to 7.8. Only the strains having potent soluble toxins were used. The filtrates were then treated with formalin, the same as in diphtheria toxoid. Dilutions were then made of 1:10, 1:100, 1:1,000 and 1:10,000. Treatment by subcutaneous injection was begun with 0.1 c.c. of the highest dilution and increased gradually depending upon the patient's reaction to the original test dose of the toxin and the patient's reaction to the therapeutic dose. No rule of thumb

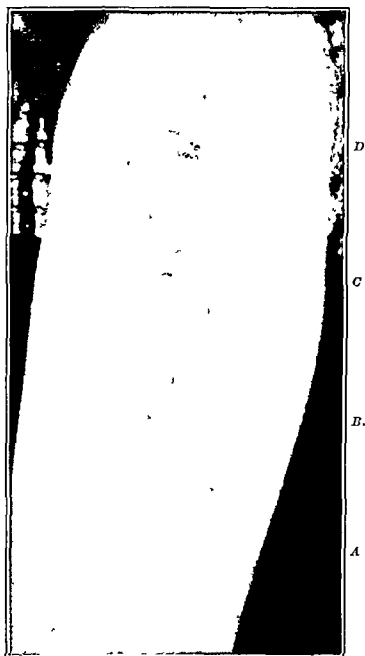


Fig. 2—Delayed reactions, twenty-four-hours A, Hemolytic streptococcus toxin B, Boiled toxin, the same as A C, Nonhemolytic streptococcus toxin D, Tuberculin 1:100 Mantoux

or standardized method of treatment has been developed so far *Individual dosage is essential and must be worked out for each patient* A very slight local reaction at the site of injection was considered favorable, and was used as a guide in raising the dosage. The intradermal tests were used as indicators of the type of streptococcus probably responsible and the one giving the strongest reaction was used in treatment, but only in single strain. Whenever possible autogenous material was used Otherwise treatment was given

The 14 patients studied presented different aspects of the disease. All of them presented marked anemia and 9 showed edema. Protein and cholesterol determinations were made in blood plasma. In 5 cases the blood was analyzed periodically for its protein content before and after treatment.

Inspection of Table I reveals a marked decrease in the amount of protein in all cases presenting edema. Lower values are found when edema is more accentuated. However, temporary high values may be observed during periods of dehydration. Old cases present lower protein values than recent ones. Acute infestation seems to be of greater influence than a chronic one in lowering plasma proteins.

TABLE I  
PLASMA PROTEINS IN PATIENTS WITH HOOKWORM ANEMIA

INTENSITY OF EDEMA	VALUES IN GR. PER 100 C.C. OF PLASMA				HEMOGLOBIN (SAHLI) %	ERYTHRO- CYTES MILLION PER 1 MM <sup>2</sup>
	TOTAL PROTEINS	ALBUMIN	GLOBULIN	FIBRINOGEN		
++	6.7	3.7	3.0	-	55	3.1
+	7.1	5.0	2.1	0.30	32	2.8
++	6.4	4.0	2.4	-	31	2.7
+++	6.5	3.7	2.8	0.48	28	2.0
+	6.6	3.8	2.8	0.47	39	1.9
+++	5.6	2.5	3.1	0.60	25	2.2
+	6.8	4.7	2.1	-	70	3.5
++	5.8	2.8	3.0	0.56	21	2.0
++	5.3	3.1	2.2	-	20	1.2
0	7.5	5.3	2.2	-	66	3.8
0	7.4	4.9	2.5	-	67	3.1
0	7.1	4.0	3.1	-	48	2.1
0	7.1	4.2	2.9	-	61	3.0
0	7.2	4.5	2.7	-	32	1.8
Average for total proteins { With edema 6.3 Without edema 7.2						
Average for albumin { With edema 3.7 Without edema 4.5						

Treatment with antihelminthic drugs associated with ferrous salts and good diet raises plasma protein with a parallel reabsorption of edema. In the absorption period some variations are observed which decrease or maintain stationary the amount of plasma proteins (Chart 1). Only after one or two months can normal values be found.

One of the most important functions of the plasma proteins is the maintenance of the physicochemical state of the blood. The fluid balance between the blood and the intercellular tissue spaces and serous cavities results on the one hand from the osmotic attraction for water, and, on the other, from the hydrostatic pressure of capillaries.

Starling, in classical experiments, demonstrated this fact by measuring by dialysis the osmotic pressure of plasma proteins. Govaerts<sup>13</sup> and Schade and Claussen<sup>14</sup> developed more accurate techniques and confirmed Starling's conclusions. Govaerts<sup>15</sup> studying separately globulin, albumin, and fibrinogen in normal human plasmas observed that albumin exerts an osmotic pressure of 7.54 cm. of water, and globulin 1.95 only. The plasma proteins, chiefly albu-

those of rheumatoid arthritis. The prognosis was given as grave. At about this stage in her clinical course, at the end of December, 1932, treatment with modified streptococcic toxin was begun and given twice weekly. After six weeks of treatment, the patient began to improve gradually, moved the joints slightly without pain, began to take nourishment better, and an increase in strength was noted. Four months after treatment was begun, the patient was out of bed on crutches and a month later she was discharged from the hospital in excellent condition without any pain in any of her joints, and walking well. She gained so much weight that the general impression was that she doubled her weight in five months. The temperature came down to normal one month after treatment was begun and remained normal until she was discharged.

CASE 2—L. E., white, female, twenty eight years old, married, was admitted to the Brooklyn Jewish Hospital on March 25, 1933, with a diagnosis of infectious polyarthritis and ulcerative colitis (18 to 20 stools in twenty four hours). The illness was of six months' duration and began with the colitis, followed a few weeks later by the arthritis. A blood study showed a marked anemia, hemoglobin, 45 per cent, red blood cells, 1,800,000, white blood cells, 16,500 with 68 per cent polymorphonuclears, sedimentation time, twelve minutes (Luzenmeier). The urine examination was negative except for a trace of albumin, complement fixation test for gonococcus was negative. The temperature ranged between 99° and 103° F daily, and she was gradually getting worse with marked emaciation, anorexia and increasing anemia. She received two transfusions, each consisting of 500 c.c. of whole blood without any effect on her downward course. When first seen by me, she had been at the hospital for six weeks, her status and clinical course being as described. The condition of the joints was as follows: all the joints were painful and tender, the joints of the fingers, wrists, elbows, knees, ankles, and feet were swollen, hot, stiff, and spindle shaped, with considerable muscular atrophy. There were beginning deformities of the hands, wrists, elbows, and knees, the latter being contracted and forming an angle of about 90 degrees with the thighs. X ray showed no destruction of joints, and the articular surfaces were intact but the spaces between them were narrowed. A study of the stool was made, and after eliminating the *B. coli* in a carbonate solution, a toxic strain hemolytic streptococcus was isolated. A toxoid was prepared, and in the meantime treatment was begun with a stock toxoid of a similar streptococcic strain, injections being given every other day, beginning with 0.1 c.c. of 1:100,000 dilution. The pain and swelling of all the joints began to diminish after the first six injections. The contractures were gradually released, and the patient regained all joint function after six weeks of treatment, which was intercepted with the autogenous toxoid as soon as it was ready. She was out of bed and began walking fairly well. Her ulcerative colitis improved at the same time to the extent that the number of stools were reduced from 18 or 20 to one or two a day, well formed. She gained considerable weight and was on a full diet when discharged from the hospital, July 11, 1933. This patient showed the most rapid change and marked improvement under this form of therapy. No other measures were used while this treatment was given, neither for the colitis nor the arthritis.

CASE 3—N. B., white, female child, eleven years old, was admitted to the Pediatric Service of the Kings County Hospital on May 2, 1933, with a diagnosis of infectious polyarthritis. Two months previously she had had an otitis media, and several weeks following this infection she began to have pains in the elbows and hips and soon after the other joints became involved. At the time of admission to the hospital, the right elbow was already ankylosed at about an angle of 90 degrees, the patient was on her back with the knees apparently ankylosed, forming an angle of contracture with the thighs of about 60 degrees. She was extremely emaciated, and all her joints were painful and stiff. She was transferred to the neurologic service as a case of poliomyelitis, but was sent back to pediatrics as a case of tuberculosis, and was then referred to orthopedics because of the marked deformities. Opinions differed from poliomyelitis to tuberculosis, to Still's disease. X ray films showed changes in the hips, knees, and elbows which were interpreted as due to an infectious process. Mantoux (old tuberculin 1:50) was negative. Blood Wassermann was negative. Repeated vaginal smears and complement fixation were negative for gonococcus. There was a moderate anemia of 50 per cent hemoglobin, 2,680,000 red blood cells, and a leucocytosis of 14,000 with 70

TABLE II

EDEMA, COLLOIDSMOTIC PRESSURE OF PLASMA PROTEINS AND CHOLESTEROL

INTENSITY OF EDEMA	COLLOIDSMOTIC PRESSURE IN CM. OF WATER (CALCULATED BY GOVAERTS' FACTOR)	TOTAL PROTEINS IN GR. PER 100 C.C. OF PLASMA	CHOLESTEROL IN MG. PER 100 C.C. OF PLASMA
++	28.4	6.7	-
+	41.7	7.1	-
++	34.8	6.4	-
+++	33.3	6.5	139
+	34.1	6.6	122
+++	24.8	5.6	122
+	38.5	6.8	152
++	26.9	5.8	94
+	27.6	5.3	120
0	44.2	7.5	133
0	41.8	7.4	142
0	36.2	7.1	103
0	36.3	7.1	151
0	39.1	7.2	86
Average	With edema	32.2	6.3
	Without edema	39.5	7.2
			124
			123

In our opinion the most important factor that affects the albumin content of plasma in hookworm disease is the state of undernutrition found in the majority of cases associated with a constant loss of blood by way of the intestinal canal. Those patients without edema and therefore with normal values for plasma proteins are well nourished, although there may be intensive intestinal infestation by parasites. When proteins are added in large proportion to the diet (beef), edema disappears and plasma proteins rise to normal level, even without antihelminthic treatment. Iron introduced by high meat diet in some cases contributes to the reabsorption of edema and in this way the patients recover a normal blood picture, according to the studies of W. Cruz.<sup>18</sup>

Bruckman and Peters<sup>16</sup> have shown that edema appears in patients with malnutrition when serum albumin is below the normal level. Wastage of serum proteins is probably the most important feature of such malnutrition. In protein-starved rats edema has been observed by Kohman, Fritsch, Mendel and Peters and more recently (in low protein diet) by Torbert.<sup>19</sup>

Edema was frequently observed during the World War due to the lack of proteins in diet and low figures in blood plasma were found (Schittenhelm and Schlichts). In beriberi cases one of us<sup>20</sup> found low plasma proteins in those patients showing edema. With recovery the edema disappears and the plasma proteins increase to normal level.

Chart 1 shows that proteinemia is also influenced in hookworm anemia by treatment and is related to the presence of edema. In two cases a slight fall in proteins was observed, probably due to the resorption of edema fluids.

The determination of cholesterol in plasma (Bloor and Knudson's technic) shows values situated toward the lower limit admitted for normal standard. In Brazil the normal average established by Villela and Silva<sup>21</sup> was 172 mg. for both sexes. The average found in 11 cases of hookworm anemia was 124 mg. per 100 c.c. of plasma. In all cases treated the cholesterol content in-

CASE 5—E G, white, male, twenty eight years old, was first seen on the Orthopedic Service of Dr L'Episcopo at the Kings County Hospital in July, 1933. The history was as follows. In 1925, he began to complain of pains in the joints of his feet and was treated at the New York Mount Sinai Hospital, where at that early stage Dr Emanuel Libman gave a bad prognosis. A tonsillectomy was done and he felt improved for about a year during which time he passed the examination and joined the U S Army. In 1926, he again developed pains in the feet, and also in the knees and hips. He was admitted to the Walter Reed Hospital and was kept there for twelve months. In November, 1927, he was readmitted to the Walter Reed Hospital on the complaint of pain in the wrists. This was a mild attack and he improved and was discharged after several weeks. In 1930 he was readmitted to the Walter Reed Hospital and this time his entire spine was involved in addition to the joints of the extremities. He was at the hospital for thirteen months and the spine was fixed. In 1932, he was admitted to the Soldiers' Home Hospital as a chronic invalid, and was to stay there indefinitely. In July, 1933, his uncle, a Brooklyn physician, wrote to him and following the communication, the patient asked to be discharged from the Soldiers' Home Hospital, came to Brooklyn, and was admitted to the Kings County Hospital for observation and study. Treatment with modified streptococcal toxin was begun at the hospital in July, 1933, and was continued after he was discharged from the hospital four months later, at first twice weekly and after six months, once a week. After ten months of treatment he made remarkable progress, and could move his neck, shoulders, hips, and knees much better, and had great relief from pain and stiffness. Occasionally he gets some pains in the back of the neck and hips on change of weather, but considering the obvious limitation of therapy in such a patient, his improvement is considered very satisfactory. He is still under observation and treatment and is seen at fairly regular intervals. His mental state of depression for many years, during which time he frequently talked of suicide, changed completely. He made good adjustment, and expressed a desire to study engineering.

CASE 6—M D, white, female, thirty seven years old married, was referred to me on Sept 19, 1933, by the late Dr W Bruce Anderson, with the diagnosis of advanced rheumatoid arthritis with marked deformities and muscular atrophy of seven years' duration. The history in brief was as follows. Pain in the joints of the feet began seven years ago, kept on with improvement and recurrences for several years and then the joints of the hands became involved. A tonsillectomy was done at this time, and she felt improved for about six months. During this period of improvement she had her first baby, and several months following the delivery, the pains recurred in the hands and feet. Two years after the first delivery she gave birth to a second baby, and following the second delivery, all the joints of the upper and lower extremities became involved. She was bedridden most of the time, and at best could sit in a wheel chair, frequently being unable to feed herself. When she was first seen by me the hands were deformed with marked atrophy of the interossei muscles, the wrists were very swollen and stiff, the right elbow was contracted at about an angle of 90 degrees, the shoulders and hips were immobile, the knees were very swollen and partially contracted and the ankles and feet were similarly swollen, the left ankle being about twice the size of normal. She could not walk, and could barely stand up, but not without support. Her blood pressure was 140 systolic and 90 diastolic, red blood cell study showed a moderate anemia, white blood cells were 6,200 with 76 per cent polymorphonuclears. Urine examination was negative, blood chemistry normal, blood Wassermann and Kahn tests and complement fixation for gonococcus were negative. Her sedimentation time (Lanzenmeier) was eighteen minutes. A complete bacteriologic survey of possible foci of infection was undertaken, and a nonhemolytic streptococcus was isolated from her nasopharynx and a similar organism from the stool. Intra dermal tests with these organisms were strongly positive and lasted for a week. An autogenous toxoid was prepared and treatments were given twice weekly. After four months of treatment the patient was free from pain, the joints were remarkably improved, she was able to walk and to travel on the street car to the office, and two months later, i.e. six months after treatment was begun, she was able to do her own housework and take care of her family. She still returns regularly about once a month for observation, and she feels entirely well.

15. Govaerts, P.: *Le Fonctionnement du Rein Malade*, Paris, 1936.
16. Bruckman, F. S., and Peters, J. P.: Plasma Proteins in Relation to Blood Hydration, *J. Clin. Investigation* 8: 591, 1930.
17. Moschcowitz, E.: Hypoproteinemia, *J. A. M. A.* 100: 1086, 1933.
18. Cruz, W.: Pathogenesis of Anemia in Hookworm Disease, II and III, *Mem. Inst. Osw. Cruz* 29: 427, 1934.
19. Torbert, H.: The Effect of Fasting on the Serum Protein Concentration of the Rat, *J. Exper. Med.* 62: 1, 1935.
20. Villela, G. G.: Protéines du Plasma, Calcium et Cholesterol dans le Beriberi, *Compt. rend. Soc. de biol.* 113: 1277, 1933.
21. Villela, G. G., and Silva, C.: Lipids of Normal Plasma, *Mem. Inst. Osw. Cruz* 27: 1, 1933.
22. Man, E. B., and Gildea, E. F.: Serum Lipoids in Malnutrition, *J. Clin. Investigation* 15: 203, 1936.
23. Bloor, W., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol-Esters in Small Amounts of Blood, *J. Biol. Chem.* 27: 107, 1916.

## SNAKE VENOM (MOCCASIN) IN THE TREATMENT OF EPILEPSY\*

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**S**TORIES of the alleged freedom from seizures of epileptics after having been bitten by snakes of the adder family were followed by enthusiastic reports by Spangler of snake venom (crotalin) treatment in epilepsy.<sup>1-4</sup> Spangler<sup>3</sup> said that not only are the virulence and number of epileptic fits favorably influenced by crotalin, but the excitability of the nervous system is modified and the general health of the patients, their mental faculties and metabolism, in every respect, are considerably improved. Anderson<sup>5</sup> reported the death of a patient under crotalin treatment and strongly urged against this therapy on the basis of the bacterial contamination of the venom then in use, and its antibactericidal action. Spangler<sup>4</sup> thought that crotalin solution was favorable in epilepsy because of its power to decrease the rate of coagulation of the blood, which he thought was increased in epilepsy. Thom<sup>6</sup> reported unfavorable effects of crotalin treatment and opposed the foundation upon which the treatment was based. He found the coagulation rate of the blood of epileptics to be within normal limits.

Of Thom's 14 cases of epilepsy on crotalin treatment, one developed hysterical attacks, in 6 the severity and frequency of convulsions increased, one, a case of hystero-epilepsy showed improvement, one had a violent local and systemic reaction, and there was no change in the rest. In 1926 Fackenheim<sup>7</sup> reported favorable results following venom treatment in epilepsy. He had been treating his patients since 1911<sup>8</sup> following the reports by Spangler. Of 50 patients he observed improvement in 60 per cent and 40 per cent were free of convulsions for twelve years. In a paper published in 1927, Spangler<sup>9</sup> limited the venom treatment to the type of epilepsy in which there is an allergic factor and did not mention his early theory of the effect of venom on the blood coagulation rate. He injected small doses of crotalin solution to

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## BLOOD CHEMISTRY IN HOOKWORM ANEMIA\*

GILBERTO G VILLELA AND J CASTRO TEIXEIRA, RIO DE JANEIRO, BRAZIL, S A

VERY few publications devoted to the study of blood chemistry in hookworm anemia have appeared in the literature. Protein determinations in two cases by Vadala<sup>1</sup> in Italy and fatty acids and total lipids by Oswaldo de Oliveira<sup>2</sup> and J Villela<sup>1</sup> in Brazil, are the only known papers dealing with this question. It can be said that most of the work done in hookworm disease is dedicated to hematologic studies. The chemical aspects, with the exception of hemoglobin, have not yet been considered. However, clinical studies have called attention to some disturbances like edema and undernutrition with which chemical changes should be correlated.

In 1929 we published our first report on the blood chemistry of hookworm anemia.<sup>3</sup> Calcium, potassium chlorides, cholesterol and alkali reserve were then studied. Calcium determinations showed normal values (80 to 108 mg per 100 c c) and potassium slight lower average (16.3 to 20.30 mg per 100 c c) than normal, probably caused by the decreased number of red corpuscles. Cholesterol was only investigated in a few cases and nearly normal figures were found, Blood Sackett's technic being employed.

The most interesting fact then observed dealt with the marked fall in the total serum protein. In a subsequent paper<sup>4</sup> (1930) this question was reinvestigated and Howe's technic was adopted for the determination of the albumin and globulin fractions and a gravimetric one for fibrinogen. These analyses showed that the fall in proteins previously observed was due to a decrease in the albumin fraction, the globulin being unaffected or slightly increased. This fact was afterward confirmed by F Ribeiro<sup>5</sup> in Brazil (1931) and by Eerkens<sup>6</sup> (1932) in Java.

In the present paper the chemical changes in blood plasma of patients with hookworm anemia are shown and a suggested correlation between these changes and edema production are given.

Normal values for plasma proteins varied from 7.0 to 8.8 gm per 100 c c (Rowe, Hammarsten, Patein, Myers, Epstein, Salvesen, Codouris, Wiener and Wiener<sup>7-9</sup>). Our normal values obtained in preabsorptive conditions have shown an average of 7.82 gm (7.0 to 7.9). Howe's technic was employed in all cases for protein fractions and Chandler's was employed for fibrinogen.<sup>10-11</sup> Linder, Lundsgaard and Van Slyke<sup>12-13</sup> reported 3.4 gm for the albumin and 2.3 to 2.9 for the globulin fraction of blood plasma. Our findings varied from 4.4 to 6.0 gm (average 5.1) for the albumin and 1.8 to 2.8 gm (average 2.4 gm) for the globulin.

\*From the Oswaldo Cruz Institute  
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patients were considerably more irritable when under venom treatment than during any other period. The severity and frequency of the seizures after discontinuing the injections of venom, both during luminal treatment and under no treatment, corresponded to the observations made during similar periods before the venom was administered.

The injections of snake venom caused local reactions in the form of erythema and swelling which subsided after cold applications.

TABLE I

PATIENT	AGE AT ONSET (YEARS)	PRES-ENT AGE	FREQUENCY OF SEIZURES DURING NO TREATMENT PERIOD (PER WEEK)	FREQUENCY OF SEIZURES DURING LUMINAL TREATMENT (PER WEEK, UNLESS OTHERWISE STATED)	FREQUENCY OF SEIZURES DURING TREATMENT WITH SNAKE VENOM (MOCCASIN) (PER WEEK)	HIGHEST EOSINOPHILIC RESPONSE IN PER CENT
H. S.	14	42	4	2 per month	5	8
R. E.	9	30	2	0	5	7
G. H.	9	32	1	2 per month	3	6
J. R.	12	35	4	1	6	8
E. H.	8	21	6	1	8	15
L. K.	16	25	5	1	7	6
M. T.	9	34	3	0	4	3
F. G.	22	28	1	1 per month	8	8

## COMMENT

It became obvious after nine weeks of treatment that snake venom therapy is of no value in institutional epilepsy. Instead of inducing a refractory state toward convulsive seizures in patients with epilepsy, it probably renders them more susceptible to fits. This is in agreement with the findings of Thom<sup>6</sup> and contradicts the reports of Spangler and others. Although this experiment was done on institutional epileptics who probably have organic brain changes, nevertheless it may be deduced that the venom does not render a patient refractory to a convulsive seizure viewed as a symptom. The reactivity of the patients to venom injections was well marked as shown by the local reaction as well as the definite response in eosinophilia. There was no correlation between the percentage eosinophilia and the effect of the venom on the frequency of seizures.

## SUMMARY

Eight institutional epileptics were treated for nine weeks with snake venom (moccasin). Injections of a 1:3000 venom solution were given, beginning with an initial dose of 0.2 c.c. and increasing by 0.2 c.c. until a dose of 1.0 c.c. was reached. The injections were given twice a week until the maximum dose was reached, when they were administered at weekly intervals. The course of treatment lasted nine weeks. A local reaction in the form of erythema and swelling at the site of injection, and a general reaction evidenced by an eosinophilia were observed. The frequency and severity of the seizures were compared during the periods of no treatment, during luminal therapy and during the administration of venom. During the administration of venom the

min, are in opposition to hydrostatic pressure of capillaries, edema appearing when the colloid osmotic pressure of protein (or "oncotic pressure" of Schade and Claussen) falls below a given limit

The clinical and physiologic studies of Epstein, Leiter, Darrow and Hopper confirmed brilliantly the theory proposed first by Staling and developed afterward by Govaerts

We suppose in our cases of hookworm anemia that edema appears consequently to a low oncotic pressure due to the marked decrease in the albumin fraction. The calculation by means of Govaerts' factors gives us low figures for the colloid osmotic pressure. A correlation between the latter and the presence of edema is shown in Table II

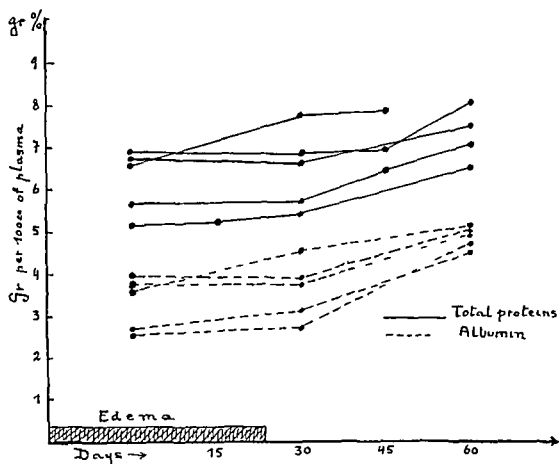


Chart 1—Plasma proteins during treatment

In malnutritional and cachectic edema low figures for plasma proteins and the relation between the nature of edema and hypoproteinemia are frequently shown as reported by many authors (Kurek and Neumann, Kohman, Mayer, Fritsch, Mendel and Peters, Bruckman, D'Esopo and Peters<sup>13 10</sup>)

In hookworm anemia a hyponutritional state is commonly found and edema can be attributed to the decrease in plasma proteins. According to Mosecowitz<sup>1</sup> three different kinds of disorders may be responsible for hypoproteinemia: loss of proteins (by kidney, digestive tube, hemorrhages), insufficient production and diet poor in proteins. In nephrosis and nephritis the wastage of proteins is due to their loss in urine. On the other hand, in hookworm disease albuminuria is slight and the decrease in plasma proteins cannot be attributed to this cause.

## DURATION OF IMMUNITY FOLLOWING DIPHTHERIA PROPHYLAXIS\*

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IN 1913, Behring<sup>1</sup> demonstrated that the injection of a properly balanced mixture of diphtheria toxin-antitoxin in man would produce no harmful effects and yet produce an antitoxic immunity to diphtheria. Since that time efforts have been made, with more or less success, to prepare products that would engender a better and more lasting immunity.

In 1923, Glenny, Allen and Hopkins<sup>2</sup> proposed that formalinized toxin, or toxoid, be used in human immunization and in 1924 Ramon<sup>3</sup> of the Pasteur Institute perfected such a preparation which, when treated with formalin and heat, completely lost its toxicity but still retained its flocculating and antigenic qualities. It was found to be much more stable than toxin-antitoxin, having greater resistance to heat and demonstrating no apparent change from freezing. One of its chief appeals was that it contained no animal serum, thus avoiding any possibility of serum sensitization following its administration.

From information available two doses of toxoid appear to be from 20 to 30 per cent more effective than three doses of toxin-antitoxin, and the immunity is produced much more rapidly.

In 1926, Glenny, Pope, Waddington and Wallace<sup>4</sup> showed that the antigenic value of diphtheria toxoid was improved by the addition of aluminum potassium sulphate and in 1931, Park and Schroder<sup>5</sup> used alum toxoid for immunization of children with very satisfactory results.

In 1932, Wells, Graham and Havens<sup>6</sup> described the complete precipitation of diphtheria toxoid with alum following the method of Glenny and Barr.<sup>7</sup>

A single injection of the alum precipitate tested in guinea pigs produced such a high degree of immunity that the observations were extended to the effects of a single injection in children.

In later publications, Havens and Gill,<sup>8</sup> Graham, Murphree, and Gill,<sup>9</sup> McGinnes, Stebbins and McCoy,<sup>10</sup> McGinnes, Stebbins, and Hart,<sup>11</sup> Massey,<sup>12</sup> Baker and Gill<sup>13</sup> and others reported on thousands of cases in which a high rapid immunity was produced by one dose of alum precipitated toxoid.

Park<sup>14</sup> introduced it in the New York City Public Schools in 1934, and found that one dose conferred immunity in 95 per cent of susceptible children.

Keller and Leathers<sup>15</sup> state that results following immunization with a single dose of alum precipitated toxoid compare favorably with those obtained after two doses of toxoid and are much better than those after three doses of toxin-antitoxin mixture.

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creased and attained normal levels. The hypocholesterolemia observed in this disease seems to be related to a diet poor in cholesterol. In well nourished cases higher values are found.

Man and Gildea (in 10 malnourished patients who were followed for some time) found that the cholesterol of plasma varied with the state of nutrition. Values below normal were found in the majority of cases studied and are usually present together with hypoproteinemia.<sup>22</sup>

The kidney injuries observed in hookworm disease and belonging to a fatty nephrosis (Ashford and Igavanesli) are not of the same kind as in lipid nephrosis and have no influence on the renal function. Hypocholesterolemia and hypoproteinemia are produced by a malnutritional state and are not related to renal disorder.

#### CONCLUSIONS

1 In hookworm anemia with edema low values for plasma proteins are frequently found.

2 Hypoproteinemia is due to the decrease in the albumin fraction only, the globulin being unaffected or slightly increased.

3 Our observations suggest that the decrease in plasma albumin must be due to a lack of protein in the diet and to the state of undernutrition frequently found.

4 Edema in hookworm disease seems to be caused by the decrease in the colloid osmotic pressure of blood proteins consequent to the fall in its albumin content. It seems to be produced by a mechanism similar to the edema observed in cases of malnutrition.

5 Low values for cholesterolemia were found and are attributed to a diet poor in cholesterol.

6 Treatment associated with rich protein diet raises the protein content of plasma and therefore contributes to the disappearance of edema.

#### REFERENCES

- 1 Vadala, A. Clin Med Ital 6 1907, quoted by Rowe. Arch Int Med 18 455 1916
- 2 Oliveira, O. Pathogenia dos edemas na Ancylostomose. Folha med 4 9, 1923
- 3 Villela, G. G., and Teixeira, J. C. Blood Study in Hookworm Anemia, Suppl. Mem. Inst Osw Cruz 6 55, 1929
- 4 Villela, G. G., and Teixeira, J. C. Plasma Proteins in Hookworm Disease, Mem. Inst Osw Cruz 27 50, 1930
- 5 Ribeiro, I. Normal Plasma Proteins, Thesis, S. Paulo, 1931
- 6 Eerkens, E. OEdeem in Indie, Batavia, 1922
- 7 Codoumis, A. La Protidémie et la Pression Osmotique des Protides. Paris 1934
- 8 Salvesen, H. A. Plasma Proteins in Normal Individuals, Acta med Scandinav 65 147, 1926
- 9 Wiener and Wiener. Plasma Proteins, Arch Int Med, p 236 1930
- 10 Howe, P. E. The Determination of Proteins in Blood—a Micro method, J Biol Chem 49 109, 1921
- 11 Chandler, J. The Determination of Fibrin in Blood Plasma, J Lab & Clin Med 12 1092, 1927
- 12 Linder, G. C., Lundsgaard, C., and Van Slyke, D. D. The Concentration of the Plasma Proteins in Nephritis. J Exper Med 39 887, 1924
- 13 Peters, J. P., and Van Slyke, D. D. Quantitative Clinical Chemistry, 2 vs., Baltimore, 1931
- 14 Schröde, H., and Clausen, F. Der onkotische Druck des Blutplasmas und die Entstehung der renal bedingten Odeme, Ztschr f klin Med 100 363, 1924

now possible to assert that immunization with toxoid precipitate is a simplified means of fighting diphtheria, and that in his experience it confers as great a protection as that produced by the Ramon toxoid.

Recently a pamphlet was distributed on the *Detailed Study on Diphtheria Immunization With Alum Precipitated Toxoid* by H. H. Pansing, Health Commissioner of Montgomery County, Dayton, Ohio, and E. R. Shaffer.<sup>18</sup> One group of 462 children, given one dose of 1 c.c. of alum precipitated toxoid, and re-Schicked twenty-eight days later, showed 388 or 84 per cent negative. The 74 Schick-positive children were retested after sixty days and seven were negative, giving a total of 85.5 per cent negative.

A second group of 495 Schick-positive children were given a single dose of alum precipitated toxoid. Approximately sixty days later 445 of these were re-Schicked and 363 or 86 per cent were found negative.

A third group consisted of children from the two groups, all of whom were Schick-negative twenty-eight or sixty days following administration of one dose of alum precipitated toxoid.

Of this group 549 were again Schick-tested two years after their toxoid vaccination and of these 317 or 57.8 per cent had reverted to Schick-positive.

The children who were Schick-positive sixty days after their original dose of toxoid were given a second injection. Of this group 105 were Schick-tested two years later and 43 or 40 per cent had reverted to Schick-positive.

TABLE III  
SUMMARY FROM PANSING AND SHAFFER'S PAMPHLET

GROUP	ALUM TOXOID	NUMBER CHILDREN	SCHICK TEST AFTER SIXTY DAYS	
			NUMBER	SCHICK NEGATIVE
A	1 c.c.	468	462	85.5%
B	1 c.c.	495	445	86.0%
C	After two years		549	SCHICK POSITIVE
	(Two doses toxoid)		105	57.8%
				40.0%

Pansing and Shaffer conclude that the evidence obtained indicates that at least three doses of any diphtheria antigen of choice are required to establish a reasonable degree of permanency.

The object of this paper is to report on the results offered in a small series of our own. A large institution in the state of Indiana with approximately 500 children between the ages of seven and twenty, and 100 adults (teachers, supervisors, kitchen help, laundry, etc.) had been having numerous cases of diphtheria each winter. In 1928 they had a particularly severe epidemic and our aid was requested.

Dr. E. G. Stewart and Jones Schick-tested and made throat cultures of every one in the institution. All the Schick-positives were made Schick-negatives by administration of toxin-antitoxin mixture.

Each year since that time (with the exception of 1933) all of the new inmates were Schick-tested and Schick-positives, treated with some form of diphtheria immunizing agent, and re-Schicked to prove establishment of immunity. Since none of them had been re-Schicked for a considerable period

induce a decreased state of allergy or nonspecific desensitization. He limited the usefulness of venom to those cases in which the patients react to the injections with an increase in eosinophile cells. The eosinophilia following venom injections is said to be an indication of the reactive power of the individual, and in cases in which there is no eosinophilic response improvement is not to be expected. Fitzsimmons<sup>10</sup> in 1929 reported successful results in epileptics treated with a mixture of venoms distributed under the name of "venene."

The favorable results reported from the use of snake venom in epilepsy and the availability of a carefully prepared and standardized snake venom (Moccasin Mulford) has led me to institute this treatment in a group of epileptics at the Elgin State Hospital. Moccasin snake venom has been used with favorable results in some hemorrhagic conditions.<sup>11</sup> There is no distinguishable difference in the physiologic action of the venom of the water moccasin from that of the rattlesnake.<sup>12</sup>

#### PROCEDURE

Eight patients with frequent epileptic seizures were selected for this therapeutic investigation. At this point it is appropriate to mention that institutional epileptics differ from extramural epileptics, not only in the fact that the former show mental deterioration but also in that they have organic changes in the brain. Although epileptics in Elgin are not classified with the organic brain disease group, it is a common experience here to find gross brain changes at autopsy, such as marked dilatation of the ventricles.

A study was made of the records of these patients as to their previous frequency of convulsions under luminal therapy and when receiving no treatment. Moreover, they were observed for a week without treatment before instituting venom therapy. Snake venom solution (Moccasin Mulford) 1:3000 was used. The initial dose was 0.2 cc injected intradermally in the upper arm. The succeeding doses were increased by 0.2 cc until the maximum dose of 1.0 cc was reached. The injections were given twice a week, and after a dose of 1.0 cc was reached, the injections were given at weekly intervals. I was guided by the eosinophilic response as to the time of the injection, the venom having been administered when the eosinophilia subsided. The therapy was continued for nine weeks. An attempt was made to discontinue luminal and keep the patients on venom alone, but it was found that the frequency and severity of the convulsions increased to an alarming extent, the patients sustaining numerous injuries during the seizures. It was deemed advisable, therefore, to include 1 ss gr of luminal, bid in the treatment. However, the patients were given venom alone, luminal having been omitted for a week at a time in the beginning, middle, and toward the end of the nine week period of treatment. The definitely unfavorable effects of the venom made it advisable to discontinue this form of treatment after nine weeks. They were again placed on luminal therapy for two weeks and were then observed for a week under no treatment whatever. The results are summarized in Table I.

The seizures were more severe and more frequent during the venom therapy period than on luminal or during the period of no treatment. The

Thirteen untreated children who were Schick-negative during October, 1934, are still negative sixteen months later. At this time (February, 1936) 70 new students have been Schick-tested. Twenty are positive and will be inoculated with one injection of alum precipitated toxoid.

TABLE IV  
REVERSION OF SCHICK NEGATIVES OVER A PERIOD OF EIGHT YEARS

	POSITIVE	TOTAL	PER CENT REVERSION
8 years previously—			
Immunized with toxin-antitoxin mixture	6	121	5.0
No history of previous immunization	3	13	23.0
6 years previously—			
Immunized with toxoid	3	21	14.3
4 and 5 years previously—			
Immunized with toxoid	4	58	6.8
No history of previous immunization	0	21	0.0
16 to 24 months previously—			
Immunized with alum toxoid	1	49	2.0
No history of previous immunization	0	26	0.0

In this series of approximately 428 children a Schick control test was made along with the Schick test.

It is interesting to note that 47 or 11 per cent gave positive pseudoreactions. Of these, 10 were positive-combined and 37 were negative Schick.

In subgrouping these 47 we find:

- 18 had no record of previous injections of toxoid or toxin-antitoxin.
- 22 had been inoculated with toxin-antitoxin mixture.
- 2 had been inoculated with toxoid.
- 5 had been treated with ointment.

While 11 per cent of the whole group gave pseudoreactions, all of those who reacted were over thirteen years of age. This would indicate the importance of a Schick control test in all individuals over twelve or thirteen years of age.

#### CONCLUSIONS

While the number of retests are small, the result would agree with those of Gill, and McGinnes, Stebbins and Hart, and Faragó, indicating that alum precipitated toxoid is a better immunizing agent than toxoid; and toxoid, in turn, is better than toxin-antitoxin mixture and that this immunity is just as lasting after alum precipitated toxoid.

#### REFERENCES

1. Von Behring, E.: Ueber ein Neues Diphtherieschutzmittel, Deutsche med. Wehnschr. 39: 873, 1913.
2. Glenny, A. T., Allen, K., and Hopkins, B. E.: Testing Antigenic Value of Diphtheria Toxin-Antitoxin Mixtures, Brit. J. Exper. Path. 4: 19, 1923.
3. Ramon, G.: Sur la Toxine et sur l'Anatoxine Diphthériques. Pouvoir Flocculant et Propriétés Immunisantes, Ann. Inst. Pasteur 38: 1, 1924.
4. Glenny, A. T., Pope, C. G., Waddington, H., and Wallace, U.: The Antigenic Value of the Toxin-Antitoxin Precipitate of Ramon, J. Path. & Bact. 29: 31, 1926.
5. Park, W. H., and Schroder, M.: Diphtheria Toxin-Antitoxin and Toxoid. A Comparison, Am. J. Pub. Health 22: 7, 1932.

frequency and severity of the seizures were greater than during the other periods and the patients were more irritable. There was no correlation between the seizures and the cosmophilic response.

It is concluded that venom therapy not only does not induce a refractory state to convulsive seizures in institutional epileptics but may render them more susceptible to seizures. Although this conclusion is based on experience with institutional epileptics, it probably is also applicable to extramural epilepsy, viewing the convulsive seizures as a symptom.

## REFERENCES

- 1 Spangler, R. H. The Treatment of Epilepsy With Hypodermic Injections of Rattle snake Venom (Crotalin). Preliminary Report, *N. Y. Med. J.* 92: 462, 1910.
- 2 Spangler, R. H. Crotalin Treatment of Epilepsy, *N. Y. Med. J.* 94: 517, 1911.
- 3 Spangler, R. H. The Crotalin Treatment of Epilepsy, *N. Y. Med. J.* 96: 520, 1912.
- 4 Spangler, R. H. The Treatment of Epilepsy With Hypodermic Injections of Crotalin, *N. Y. Med. J.* 97: 689, 1913.
- 5 Anderson, J. F. Danger in the Subcutaneous Injections of Solutions of Crotalin, *I. A. M. A.* 62: 893, 1914.
- 6 Thom, D. A. The Present Status of Crotalin in the Treatment of Epilepsy, *Boston M. & S. J.* 171: 933, 1914.
- 7 Fackenheim, S. Die Wirkungen und Erfolge der Krotalinbehandlung der genuine Epilepsie, *Deutsche Ztschr. f. Nervenheilk.* 94: 124, 1926.
- 8 Fackenheim, S. Neue Wege zur Heilung der Epilepsie, *München med. Wchnschr.* 58: 1872, 1911.
- 9 Spangler, R. H. Allergy and Epilepsy, Analysis of One Hundred Cases, *J. Lab. & Clin. Med.* 13: 41, 1927.
- 10 Fitzsimmons, F. W. Snake Venoms: Their Therapeutic Uses and Possibilities. Read at the British and South African Association for the Advancement of Science, Cape Town, July 24, 1929.
- 11 Peck, S. M., and Rosenthal, N. Effect of Moccasin Snake Venom (*Ancistrodon piscivorus*) in Hemorrhagic Conditions, *J. A. M. A.* 104: 1066, 1935.
- 12 Essex, H. E. The Physiologic Action of the Venom of the Water Moccasin (*Agkistrodon piscivorus*), *Am. J. Physiol.* 99: 681, 1932.

be lipoidal in nature and probably is not thoroughly wet by the blood plasma. The fact that erythrocytes do not adhere in the normal blood stream indicates that the surface tension effect is not very pronounced, since corpuscles frequently come in contact with others.

The forces due to surface tension, when they operate on two erythrocytes already in contact, undoubtedly do have a tendency to place them face to face; this is the position of least overall surface.<sup>2</sup> But if a pile of corpuscles has already formed in rouleau, it would be anticipated that a considerable proportion of future additions to the pile would become attached to the sides if the contacts were entirely adventitious. Observation shows that this rarely happens. It is accordingly apparent that a force operates within the liquid to place the free erythrocytes on the ends of the rouleau piles which are already formed.

Erythrocytes are only about 5 per cent heavier than the blood plasma which they displace. Their movements accordingly respond to very delicate forces; they are like feathers floating in air. Also, the erythrocytes compose about one-fourth of the volume of the blood, and they are accordingly relatively numerous and only slightly separated from one another.

The formation of the rouleaux appears ordinarily to take place just as the cover glass settles into position before the blood coagulates. The placing of the cover glass on the drop of blood causes a relatively strong current of blood to flow. The piling of red blood corpuscles into rouleau formation may be greatly increased by pumping the blood back and forth under the cover glass by means of intermittent pressure, such as could be exerted with a pencil point. Streams of the viscous plasma pass about the corpuscles whenever the conditions of movement are changed or reversed, because of the greater inertia of the corpuscles which are slightly heavier than is the plasma. Hydrodynamic reasoning indicates that the distribution of the velocity of the moving plasma is a maximum halfway between the plates. Further, and particularly important, the corpuscles which are immersed in the moving liquid tend to occupy the center of the stream and assume a position of maximum stability, which is with their plane surfaces at right angles to the stream flow, i.e., with their axes of symmetry parallel to the stream flow. This is the condition of maximum stability as was shown by the physicist Lord Rayleigh who mathematically analyzed the conditions of flow about a disk in a stream of viscous fluid. He showed that the internal friction of the fluid as it flows around the edges of the disk causes a couple to be set up. The torque exerted by the couple places the plane of the disk at right angles to the stream flow. All the discoid corpuscles tend to do this and accordingly there is an orderly procession of them with their broad sides parallel; and if they come together at all, it is always in this broadside-parallel fashion. The relative motion between the corpuscles and the plasma is such that the resistance of the flow of the liquid about the erythrocytes is decreased after the rouleaux have formed. These are then the causes which set up the formation of rouleaux. Hydrodynamic forces operate to bring the corpuscles together with their faces parallel and the forces of surface tension complete the positioning of the corpuscles and hold them together.

## DURATION OF IMMUNITY

On the basis of guinea pig experiments, carried on for a long period of time, it was presumed that in human subjects an immunity equal to or superior to that obtained with regular toxoid could be obtained with alum precipitated toxoid

Gill, quoted by Walker,<sup>16</sup> reported that in a retest of forty of his original group of children made two years later, there was one that gave a slightly positive reaction, all the rest were negative. All of these children were strongly Schick positive before receiving the alum precipitated toxoid, and all had reacted negatively after injection.

McGinnes, Stebbins, and Hart<sup>11</sup> reported on the durability of immunity produced by one dose of alum precipitated toxoid at the end of one year (Table I)

TABLE I  
TABLE FROM MCGINNES, STEBBINS, AND HART  
Am J Pub Health 24 1141, 1934

REVERSION OF SCHICK NEGATIVES IN ONE YEAR			
	POSITIVE	TOTAL	PER CENT SHOWING REVERSION
Originally Schick negative With no history of previous immunization	36	500	6.5
Originally Schick negative With history of previous immunization	24	519	4.6
Originally Schick positive Rendered Schick negative by Park's toxoid	10	225	4.4
Originally Schick positive Rendered Schick negative by alum ppt toxoid	19	342	5.6
	89	1,636	5.4

Their conclusion is that there is not a significantly greater reversion to the Schick positive state in individuals rendered Schick negative by alum precipitated toxoid than in (Schick negative) individuals rendered Schick negative by natural causes or following other diphtheria immunizing agents.

Farago<sup>17</sup> conducted some experiments in Hungary with a single dose of alum precipitated toxoid. Of a group of 2,652, 93.3 per cent were Schick negative after two months. One year later 2,379 of this same group were again Schick tested and 93.7 per cent were negative.

TABLE II  
SUMMARIZED TABLE OF FARAGO'S PAPER  
F Farago Am J Hyg 22 490, 1935

IMMUNIZED SCHICK CHILDREN		IMMUNIZING DOSE IN UNITS	RESULTS OF SECOND AND THIRD SCHICK TESTS			
AGE YEARS	NUMBER		AFTER TWO MONTHS		AFTER ONE YEAR	
			NUMBER PRESENT	SCHICK NEGATIVE	NUMBER PRESENT	SCHICK NEGATIVE
2-12	4,297	14.4 to 29.4	2,652	93.3%	2,379	93.7%

Farago states that the total number of children in Hungary immunized with toxoid precipitate, up to the end of 1934, was about 60,000, that it is

## HUMAN INFECTION WITH MONILIA\*

### REPORT OF A CASE WITH CULTURAL DATA

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**P**ATHOLOGIC processes in the human body associated with monilia are not rare, but the unusual lesions produced in this case are of interest because of the confusing similarity which caused diagnoses of syphilis, tuberculosis, and leprosy to be made before the true causative organism was found. The closed subcutaneous and the open pulmonary lesions in this case afforded an unusual opportunity for cultural studies.

E. R., a colored male, aged forty-one years, was admitted to the National Leprosarium, July 12, 1934, with a diagnosis of leprosy which could not be confirmed; therefore a study of his case was begun.

The patient was born in Virginia but had spent most of his life in Ohio. His occupations had been chiefly manual labor. The previous and family histories were not remarkable.

*Previous Illnesses:* Chickenpox at the age of four years with good recovery. Smallpox at the age of sixteen with good recovery.

About 1920 a tumor appeared on the upper lid of the left eye about the size of the end of the small finger. About two weeks after this was aspirated with a syringe, a burning of the eye was noticed, and at this time the patient noticed his vision was beginning to be blurred. A discharge continued from the eye and in spite of medical treatment the vision continued to be blurred and the eye went totally blind about 1930. At the present time the vision of the right eye is periodically blurred. The patient gave a history of having had double pneumonia in 1924 with poor recovery. The patient said he had never felt well after the attack and had retained a residual cough. Slight improvement was noted following a period of rest. During this attack of pneumonia the patient lost 23 pounds of weight, some of which was regained but he was unable to hold it. He has not gained enough strength since to do manual labor. Several years ago the patient said he had night sweats for about one week but had not been troubled with them since.

In 1924, the patient stated that soft subcutaneous tumors appeared on the anterior surfaces of both wrists, which had gradually enlarged. In 1930 small raised nodules appeared in the skin of the face, one at a time. This same year one of these nodules was removed surgically and shortly afterward several appeared within the same area. These lesions have remained practically the same until the present time.

In 1927, the patient gives a history of mashing his right index finger with a wrench and crushing the bone. Recovery was fairly good. Three years later the finger became swollen and reddened at this place. No pain was associated with the swelling at any time. It has persisted and gradually become shortened.

The patient gives a history of having had gonorrhea at the age of eighteen years and a "haircut" on the penis in 1918 but denied secondary manifestations of syphilis.

\*From the National Leprosarium.

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of time the opinion was held, after reading Pausing and Shaffer's paper, that this would be desirable to see what information on the development of immunity they would yield

Of the group of children who received toxin antitoxin in 1928, 121 were still in the institution (1936) These were 10 Schick and 6 individuals or 5 per cent were again positive

Of 13 children who were Schick negative in 1928 and who were not given any toxin antitoxin, 3 individuals or 23 per cent were positive

In 1929, several reports were published on the production of immunity to diphtheria by injection of the toxin antitoxin in a lanolin or jelly base

A number of Schick positive children were given five injections in a lanolin base The response was weak Three months later five more injections in a jelly base were applied Through an oversight a 10 Schick was not made Thirty five of these children are still in the institution and were Schick tested Fifteen or 43 per cent were still positive

During 1930, 1931 and 1932 all Schick positive children were made Schick negative with diphtheria toxoid Of those remaining

21 from 1930 3 or 14.3 per cent were again positive  
28 from 1931 2 or 7.1 per cent were again positive  
30 from 1932 2 or 6.7 per cent were again positive

Ten children from 1931 and eleven from 1932 who were negative at that time and received no toxoid, are still negative

In 1934 we started giving alum precipitated toxoid, 18 were still in the institution and after two years all are Schick negative

Thirteen who were negative in this year and received no treatment are still negative

In October, 1934 we again used alum precipitated toxoid on the new pupils who were Schick positive Forty four children were tested and 31 were found to be Schick positive These were divided into two groups 16 were given one dose of 0.5 cc containing 25 Lf units The remaining 15 were given one dose of 0.5 cc containing 15 Lf units

These children were again tested four weeks after the alum toxoid Of the children receiving 25 Lf units two or 12.5 per cent were still Schick positive of those receiving 15 Lf units 3 or 20 per cent were still Schick positive

All of these children were again tested this year (sixteen months after one injection of alum precipitated toxoid) Two were found to be Schick positive One of these was a child who was Schick positive last year on retest after receiving 15 Lf units and therefore is not a reversion The other four children who were still Schick positive four weeks after the injection of the alum toxoid are now Schick negative

The other positive reaction was a slight reaction, 13 mm in diameter, in a child who received 25 Lf units and was Schick negative four weeks after inoculation

A few firm subcutaneous nodules not over 1 cm. in diameter could be felt on the anterior surface of the left forearm. There was a small scar on the glans penis. A right inguinal hernia was present. The left eye was blind and the conjunctiva of the right eye was reddened. The heart was very rapid on slight exertion. Both lungs showed bronchovesicular breathing throughout. No râles could be heard. The physical examination was otherwise negative.

*X-ray Examination:* Lungs: Both showed dense shadows at the roots and shadows characteristic of fibrosis extending to the periphery involving all lobes. The picture was not unlike that of chronic tuberculosis. Hands: The distal and middle phalanges of the index finger of the right hand showed advanced absorption of the bone with only a slight amount remaining. The end of the proximal phalanx of this same finger showed osteoporosis and erosion. There was a slight amount of erosion of the middle phalanx of the middle finger. Similar changes were present in the middle and ring fingers of the left hand.

*Laboratory Examinations:* Urinalyses were negative except for a few granular casts. The blood counts were not remarkable. Kolmer-Wassermann tests were 4-plus and the Kahn test was 4-plus.

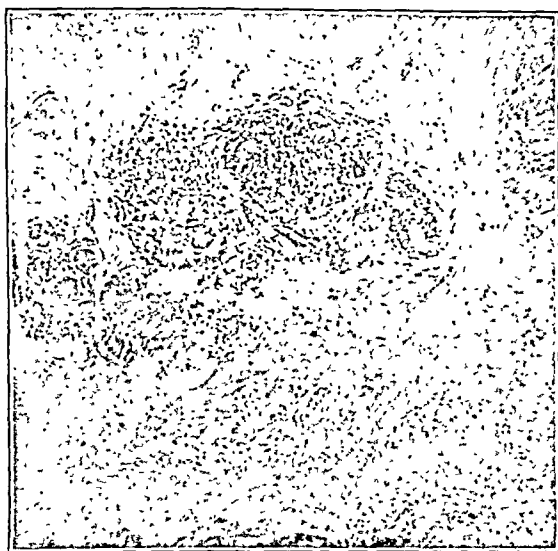


Fig. 2.—Photomicrograph of a nodule on the face, showing an area of round cell infiltration with a foreign body giant cell around a sweat gland (low power).

The sputum and nodules on the face were searched numerous times for acid-fast bacilli but none were found. Concentrated specimens of sputum were negative for acid-fast bacilli as were guinea pig inoculations and cultures on Petroff's medium. The sputum was purulent in character and blood streaked.

While searching the sputum for acid-fast bacilli frequent yeastlike organisms were seen in the smears. These cells were found to be a constant finding. A similar organism was then found in smears prepared from the papules of the face. One of the soft subcutaneous tumors of the wrist was removed and a similar organism was found to be present. This tumor consisted of a rather soft lobulated mass 2 cm. in greatest diameter, grayish yellow in color, very moist but containing no pus. Cultures of the yeastlike organism were obtained on Sabouraud's medium from the sputum and the tumor from the wrist and will be described in detail. Dark-field examinations of the sputum for spirochetes were negative in each instance.

Histologic sections prepared from the tumor removed from the wrist showed myxomatous type tissue composed of spindle cells and very loose fibrils with spaces between them. Interspersed with these fibrils were areas of scanty infiltration by lymphocytes and a few monocytes.

- 6 Wells, D M, Graham, A H, and Havens, L C Diphtheria Toxoid Precipitated with Alum Its Preparation and Advantages, *Am J Pub Health* 22 648, 1932
- 7 Glenny, A T, and Barr, M The Precipitation of Diphtheria Toxoid by Potash Alum, *J Path & Bact* 34 131, 1931
- 8 Havens, L C, and Gill, D G A Study of the Value of a Single Injection of Precipitated Toxoid in the Control of Diphtheria, *J M A Alabama* 2 328, 1933
- 9 Graham, A H, Murphree, L R, and Gill D G Diphtheria Immunization With a Single Injection of Precipitated Toxoid, *J A M A* 100 1096, 1933
- 10 McGinnes, G F, Stebbins, E L and McCoy, G W Editorial The One Dose Precipitated Toxoid, *J M A Alabama* 3 48, 1933
- 11 McGinnes, G F, Stebbins, E L and Hart C D Experience With Alum Precipitated Toxoid in Virginia and Observations on the Reaction Following Its Use, *Am J Pub Health* 24 1141, 1934
- 12 Massey, B Letter to the Editor, *Am J Pub Health* 23 948, 1933
- 13 Baker, J N, and Gill, D G Precipitated Toxoid as an Immunizing Agent Against Diphtheria, *Am J Pub Health* 24 22 1934
- 14 Park, W H A Consideration of the New Preparation of Diphtheria Toxoid, *Am J Dis Child* 47 929, 1934
- 15 Keller, A E, and Leathers, W S Alum Precipitated Diphtheria Toxoid The Rapidity of Immunization Following One Dose *J A M A* 103 478, 1934
- 16 Walker, A A One Dose Alum Toxoid in Diphtheria Immunization Chairman's Address, *J A M A* 103 227, 1934
- 17 Faragó, F Immunization Against Diphtheria, Experiments in Hungary With a Single Dose of Precipitated Toxoid *Am J Hyg* 22 495 1935
- 18 Pansing, H H, and Shaffer, E R Detailed Study on Diphtheria Immunization With Alum Precipitated Toxoid Pamphlet, 1936

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### ROULEAU FORMATION\*

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IT SEEMS that rouleau formation was first noted by "Dr Thomas Hodgkin and Joseph Jackson Lister in 1827" Both of these names are known in medicine J J Lister was the father of the late Lord Lister, who introduced aseptic surgery, and Thomas Hodgkin first described the disease which bears his name "To those observers the microscope revealed the fact that if a minute drop of human blood is placed between two plates of glass, the red corpuscles apply themselves to each other by their concave surfaces in such a manner as to form long cylindrical masses which resemble piles of coins and that very frequently those piles are so arranged as to form with each other a complete network of rouleaux with clear intervening spaces occupied by liquor sanguinalis"<sup>1</sup>

It is evident that some force must operate to bring the corpuscles together and that the same force or an entirely different set of forces must complete and maintain the rouleau formation after contact between corpuscles has once been made The last part of the suggested analysis has received considerable attention but no explanation or suggestion has been found in the literature as to the causation of the bringing together force, except for vague references to the existence of a force of attraction and the analysis of chance encounters

Surface tension, adhesion, cohesion, and similar physical agencies operate only as contact is made and exert no appreciable force between discrete particles suspended in a liquid The outer surface of an erythrocyte appears to

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capsule around the parent cell and a thinner one around the budding cells. The cells stained very irregularly, some showing many dark staining granules or masses and some almost unstained.

In cultures on Sabouraud's or dextrose tartaric acid agar, the growth was luxuriant, pearly white, moist, smooth, and rounded in outline. As the culture became older, there was a tendency for it to become dry, wrinkled, and slightly cream colored. After the organism had been isolated for several months, filaments were seen projecting from the undersurface of the colony into the media in cultures a week or more old. No aerial mycelium was produced. The original cultures made from the patient required about three days at 37° C. to develop. Later the organism grew luxuriantly in twenty-four hours at 37° C.

Microscopically, the cells in young cultures looked much as they did in tissue; the majority were oval or rounded, about 8 microns in diameter, giving off smaller budding forms. They varied in size depending to some extent on the moisture of the culture. Usually there was only one bud to a cell but as many as three or four were sometimes seen.

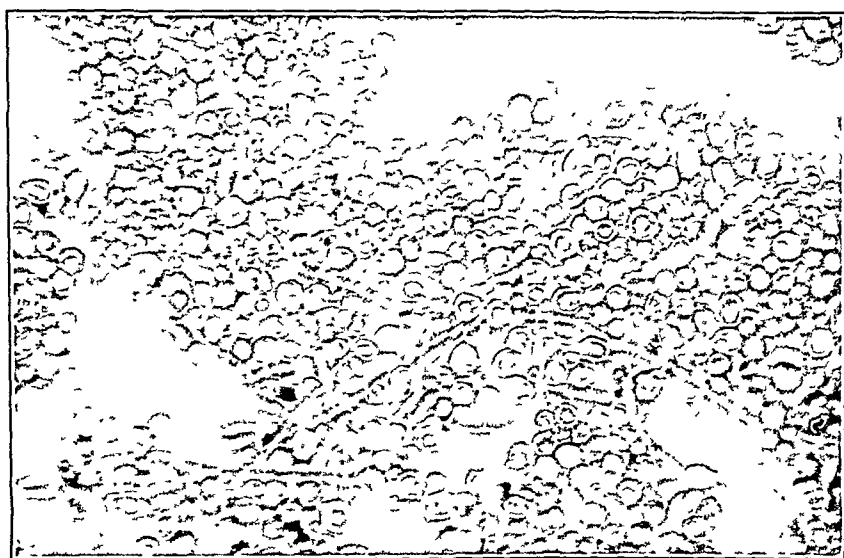


Fig. 5—Photomicrograph of a smear from the culture of monilia showing budding and mycelium formation ( $\times 600$ ).

In older cultures, on some media more than others, septate mycelia were seen giving off lateral branches and buds and terminal buds. Very occasionally terminal chlamydo spores were seen. Budding cells predominated even in smears from old cultures.

The structure of the cells could be best seen in preparations mounted in 3 per cent sodium hydroxide solution. The rounded or oval cells appeared as typical yeast cells. The cells were surrounded by a thick capsule and the budding cell by a thinner one. Vacuoles were numerous in most of the cells.

Preparations stained with 1 per cent neutral red showed the red dancing body and pink granules of typical yeast cells. Fat in the vacuoles was demonstrated by staining with Sudan III. The organism was gram positive when stained by Gram's method, and some cells contained granules more intensely stained than the rest of the cell. Some cells were stained reddish brown with Gram's iodine, others were almost unstained.

A small irregular nucleus usually at one side of the cell could be seen when the organism was stained by Mallory's chloride of iron hematoxylin method.

Richard Norris from whom a previous paragraph was quoted produced the phenomenon of rouleau formation in 1868 with water and weighted cork disks, which had been paraffined. The author has used disks 8 mm in diameter and about 1 mm thick made of celloids to study the phenomenon. Rouleaux were produced by placing a goodly number of these disks in about a centimeter of kerosene in a flat bottomed dish and gently shaking them. In such experimentation it is readily apparent that the relative motion of the liquid and the disks exerts a great influence in bringing the corpuscles together.

It has been shown also that small light particles in a medium which moves at a different velocity than they do sometimes exhibit a quite striking and even similar phenomenon to rouleau formation. See, in almost any text book of physics, descriptions of the Kundt tube method of determining the velocity of sound. Very light particles of cork dust are made to pile up in discrete masses in a tube by the induced air currents in such a way that the wave lengths of sound are readily apparent. Also, Bergen Davis has noted that light cylindrical particles like the capsules which are used in powder medication, arrange themselves end on in transverse rows across a horizontal organ pipe when it is sounding.<sup>3</sup>

#### SUMMARY

Rouleau formation of erythrocytes between a cover glass and a glass slide has been explained on the basis of hydrodynamic principles. A means is pointed out by which enhancement of the phenomenon is possible. A method for artificially producing rouleau formation with large plainly visible discoid particles is described.

#### REFERENCES

- 1 Norris, Richard. On the Laws and Principles Concerned in the Aggregation of Blood Corpuscles Both Within and Without the Vessel, Proc Royal Soc London 17: 429, 1868-1869.
- 2 Heidenham, Dr. Martin. Geldrollenbildung, Handbuch der Anatomie des Menschen, Bardeleben 8: 1067, 1911.
- 3 Davis, Bergen. On the Behavior of Small Closed Cylinders in Organ Pipes, Am J Sc 12: 185, 1901.

No ascospores were seen in preparations made from twenty-four-hour cultures on Gorodkows medium in either glass or clay petri dishes at 20° C., 31° C., or 37° C., or in preparations made from cultures on any other media.

Scant growth was obtained on Sabouraud's medium, and dextrose tartaric acid medium made anerobic either with or without the presence of carbon dioxide. More mycelial forms were seen under the microscope than in similar aerobic cultures, but the rounded or oval budding forms were also seen.

Growth was more luxuriant at 37° C. than at room temperature. Microscopically there was no difference in the morphology of the organism, whether it was incubated at room temperature or at 37° C.

*Pathogenicity:* The organism was pathogenic for rabbits, rats, guinea pigs, and mice. A suspension from a twenty-four-hour-old culture on dextrose tartaric acid medium was made in 0.85 per cent sodium chloride solution and 1 c.c. was injected intravenously into a rabbit, 1 c.c. intraperitoneally into a white rat, 1 c.c. subcutaneously into a guinea pig, and 0.5 c.c. subcutaneously into a mouse.

TABLE I

FERMENTATION REACTIONS OF *MONILIA* ISOLATED FROM PATIENT E. R.

CARBOHYDRATES, GLUCOSIDES OR ALCOHOLS	ACID	GAS
d-Glucose	+	+
d-Fructose	+	+
d-Mannose	+	+
d-Galactose	+	?
l-Xylose	+	-
l-Arabinose	+	-
Rhamnose	+	-
Maltose	+	+
Trehalose	+	+
Saccharose	+	-
Lactose	+	-
Aesculin	+	?
Dextrin	+	?
Salicin	+	-
Melezitose	-	-
Melibiose	+	-
Raffinose	-	-
Glycerol	-	-
Adonitol	-	-
Inositol	+	-
Dulcitol	+	-
d-Mannitol	+	-
d-Sorbitol	+	-

NOTE: + indicates positive, - indicates negative, and ? indicates a trace.

The rabbit died in two days. The liver presented a few well-defined abscesses filled with yellow pus and the kidneys were slightly mottled in appearance. The other organs showed no definite lesions. Smears made directly from the heart, blood, lungs, spleen, liver, and kidney showed the yeastlike organism, while the liver and kidney also showed the presence of *Eimeria stiedae* which no doubt accounted for the lesions. Smears from the meninges were negative.

The organisms were obtained on cultures made on Sabouraud's and dextrose tartaric acid media from the liver, spleen, heart blood, and kidney. A suspension made from the culture of the organism from this rabbit was injected into a second rabbit. The second rabbit died in three days and the findings were the same. Lesions in the kidney and liver were accounted for by finding *Eimeria stiedae*. Direct smears showed also the presence of the yeastlike organism in budding form in the heart blood, peritoneal fluid, lungs, kidney, and liver. Cultures from several organs were obtained on Sabouraud's and dextrose tartaric acid media. The meninges appeared normal and no yeastlike organisms were seen on direct smear.

The rat died nineteen days after intraperitoneal inoculation. A yellowish mass in the omentum about 1.5 by 1 by 1 cm. adhering to the pyloric end of the stomach was found.

The present illness dated back ten years by the patient to the attack of pneumonia. The chief complaints at the time of admission were pain in the chest, hoarseness, and cough.

*Physical Examination* The patient was emaciated, weighed 130 pounds (peak weight had been 165 pounds), was extremely hoarse and showed considerable dyspnea. The temperature range was 37° to 38° C and the pulse from 100 to 120.

The face showed small papules and nodules on the cheeks, nose, lips, forehead, and eye lids. These ranged in size from 0.2 to 1 cm in diameter and showed a tendency to mass together. The tops were excoriated and many showed umbilication with depressed centers. Some of these lesions showed redness while others showed practically no color change except the excoriated tops. The lesions were fairly firm and showed no tendency to discharge pus, although in some of the excoriated tops there was evidence of superficial secondary infection,



Fig 1—Photograph of Patient D. R. showing nodular and papular lesions of the face

but in these instances they were dry and crusty. The left cheek was free from these lesions but showed several small lesions of dermatosis papulosis nigra. The skin of the rest of the body was free from lesions, except for a few leucodermic areas on the anterior chest.

A generalized lymphadenopathy was present. Both wrists showed soft subcutaneous tumors 2 to 4 cm in diameter on the anterior surfaces which appeared to be connected with the tendon sheaths.

The right index finger was swollen and red in color. The two terminal phalanges were shortened. The nail was attached but deformed. The skin was excoriated at the juncture of the proximal and middle phalanges, and the surface was moist, although no frank pus could be expressed. The terminal phalanges of the right middle and left index fingers were "crooked."

The pathogenicity of the strain isolated was not of much value in classifying it. Spring<sup>4</sup> reports in a study of seven strains that blastomyces is more pathogenic for mice than for rabbits, guinea pigs, and rats; this organism was less pathogenic for mice. Smith<sup>5</sup> states that *Monilia psilosis* produces a fatal septicemia in rabbits in twenty-four to seventy-two hours. This organism also did but the fermentation reactions were not in accord with any of the strains as given by Smith.

The organism appeared to attack the omentum most frequently but apparently a predilection for the omentum of an animal is not a characteristic of any particular fungus. Spring found involvement of the peritoneal surface in many of the animals injected with *Blastomyces dermatitidis*. Nodules were found by Rewbridge, Dodge, and Ayers<sup>6</sup> in the omentum and the peritoneal surface of mice injected with a strain of *Endomyces capsulatus* isolated from a meningitis case. Nodules were produced almost invariably in the omentum of white rats injected with *Phialophora verrucosa* from a case of chromoblastomycosis by Wilson, Hulsey and Weidman.<sup>7</sup> Connor<sup>8</sup> reports small tubercular lesions in the omentum of mice by a monilia from a case of osteomyelitis. Benham<sup>9</sup> produced gelatinous nodules in the omentum, spleen, and lungs of white rats by injecting *Cryptococcus hominis*.

Although the names *Oidium* and *Monilia* are used interchangeably in the literature the differentiation is usually made on the basis of reproduction. The oidia give rise to new cells by a breaking up of the mycelia, giving the appearance of chains; the monilia give rise to new cells by means of budding from the mycelium. By such a basis of differentiation the present organism is a *Monilia*. This corresponds to the Blastosporinae in Vuillamin's classification.

It produced mycelia scantily as distinguished from fungi which produce them abundantly. No aerial mycelia were observed. This characteristic since it did not produce a heavy dry pellicle on liquid media would place it as a *Parasaccharomyces* according to Anderson.<sup>8</sup>

It belongs to the fermenting group as distinguished from the nonfermenting.<sup>10</sup> It might be classed as *Monilia pinoyi* according to Castellani's<sup>11</sup> classification of *Monilia* based on the production of gas from carbohydrates, although it sometimes produced a trace of gas from galactose and dextrin. Amygdalin and erythrite were not tested. As far as production of gas from carbohydrates was concerned it was also similar to a monilia isolated by Smith and Sano<sup>12</sup> from a case of meningitis. In addition to some of the carbohydrates used by Castellani, they employed mannose which their strain of *Monilia* fermented with the production of gas. It also agreed in the production of gas with the fermentation reactions of a monilia isolated from a case of osteomyelitis by Connor.<sup>8</sup>

The present organism could be called a *Monilia*, *Blastosporina* or *Parasaccharomyces*. The name *Monilia* appears to be most widely used.

#### COMMENT

That this case was proved to be an infection with monilia by cultural and animal experimentation there seems to be no doubt. Such conditions are probably not as rare as the number of diagnoses would lead one to believe.

In this case the signs and symptoms, loss of weight, cough, blood-streaked sputum, characteristic x-ray findings, and permanent hoarseness pointed to pulmonary tuberculosis. The absence of acid-fast bacilli in the sputum by smear, culture, and animal inoculation, and the presence of monilia in smears and culture which were pathogenic for ordinary laboratory animals seem to be conclusive proof that an infection of the lungs with monilia was present.

Serologically, the patient was syphilitic as evidenced by a four-plus Wassermann and Kahn test. No characteristic clinical signs of syphilis could be found, and the sputum was negative for spirochetes by dark-field examination. Clinical syphilis of the lungs is reported to be very rare by practically all observers, and with the finding of monilia in the sputum it seems reasonable to exclude syphilis as being responsible for the lung lesions.

Sections prepared from a nodule removed from the face showed slight pressure atrophy of the epidermis. The corium showed numerous circumscribed areas of infiltration of lymphocytes and monocytes with an occasional foreign body giant cell. There was slight fibroblastic reaction. There was no necrosis.

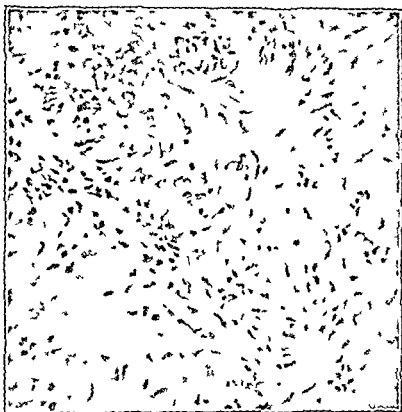


Fig. 3—Photomicrograph of a subcutaneous tumor near the wrist showing its myxomatous nature (high power)

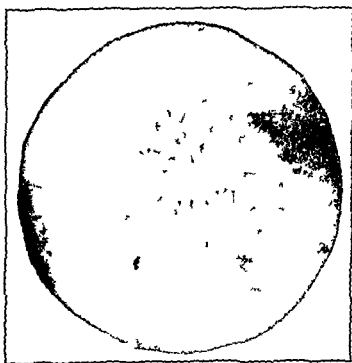


Fig. 4—Photograph of a single colony of monilia isolated from the patient and growing on starch agar. Submerged mycelium growing out from smooth moist surface colony.

**Morphologic and Cultural Characteristics** In smears made from fresh lesions from inoculated animals and stained with methylene blue or preferably with Wright's stain, the organisms appeared often in large numbers as rounded or slightly oval cells of varying sizes, but averaging about 8 microns in diameter. There was a heavy refractile, usually unstained

## BACTERIOPHAGE THERAPY IN BACILLARY DYSENTERY\*

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### INTRODUCTION

THE therapeutic value of bacteriophage in bacillary dysentery has not yet been established. Diversity of opinion as expressed in the literature may be due, in part, to the fact that some of the early reports were based upon treatment carried out in places where only meager laboratory facilities existed. These reports must be evaluated with due consideration for the three variables present in the clinical application of bacteriophage therapy to any infection: the virulence of the invader, its susceptibility to lysis by phage, and the quality of the phage employed with respect to potency and polyvalence. Failures have occurred in some instances possibly on account of delay in the use of adequate amounts of phage.

### LITERATURE

D'Herelle,<sup>1</sup> in 1921 and Da Costa Cruz in 1923<sup>2</sup> and 1924<sup>3</sup> reported favorable results in treating Shiga dysentery with 2 c.c. of bacteriophage. No mention was made of *in vitro* susceptibility tests in either instance. In 1924 Spence and McKinley<sup>4</sup> treated Shiga and Flexner dysentery with a phage, isolated from a stool filtrate. Twenty patients were treated, nineteen within the first week of illness, with 10 c.c. of this phage given by mouth three times a day. The mortality rate was 10 per cent and the average period of hospitalization 5.8 days. A control group in another hospital, given no phage, had a mortality rate of 40 per cent, and an average period of hospitalization of 12.8 days. In Egypt, Compton,<sup>5</sup> in 1929, reported favorable results and a decreased death rate in treating 200 cases of bacillary dysentery. He used a phage obtained from d'Herelle together with three races isolated in Egypt, and gave a total dose of 6 c.c. Choudhury and Morison<sup>6</sup> in 1929 treated 80 cases of Flexner and Shiga dysentery with a polyvalent bacteriophage, giving 2 c.c. three times a day the first day, and twice a day thereafter. Because of limited laboratory facilities, no susceptibility tests were made. The mortality rate was only 4 per cent.

In contrast to these apparently favorable results, Davison<sup>7</sup> in 1922 reported a mortality of 58 per cent in a group of 12 children treated with bacteriophage. Dysentery bacilli (Flexner type) were isolated from the stools of 10 of these patients. Of the 8 strains tested *in vitro*, 7 were susceptible to the phage used. Comparatively large and frequent doses were given, the total dose ranging from 5 c.c. to 1,381 c.c. Seven patients were treated with phage orally and 5 by enema. Failure in this instance was attributed to the

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An acid reaction of the medium was most favorable. Growth on nutrient agar of pH 7.0 was less luxuriant than on an acid medium of pH 5.0 to 3.8. When the sputum and tumor were cultured on Petroff's medium, no growth occurred although future transplants showed growth on this medium.

In bouillon the organism grew in the bottom of the tube as a scant granular sediment when first isolated. Later, it grew more abundantly as a coarsely flocculent mass near the surface, which soon sank to the bottom and became granular.

In 1 per cent peptone in half molecular soluble phosphate solutions Draper<sup>1</sup> found that *Odium albicans* produced abundant mycelium. This organism however, in 1 per cent peptone in one half molecular sodium acid phosphate solution grew only as a granular sediment on the bottom and sides of the tube. Microscopically the round or budding cells predominated, but there were a few mycelial forms giving off budding cells on the ends. Growth in milk was poor. Lead acetate agar was blackened. Litmus was not reduced. Indol was not produced.

Gelatin was not liquefied and growth in this medium was scanty. No filaments radiated out from the stab line and no arthrospores were seen when the organisms were examined microscopically.

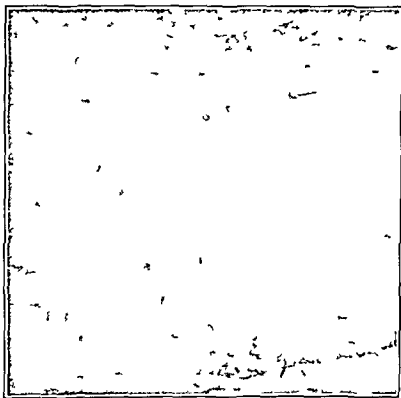


Fig. 6.—Photomicrograph of an inflammatory tumor mass of the omentum of a rat produced by an intraperitoneal injection of the monilia. Note foreign body giant cells (low power).

Fermentation reactions were tested by growing the organism in 1 per cent peptone and 0.8 per cent sodium chloride solution containing 2.5 per cent of the different carbohydrates, glucosides or alcohols with brom thymol blue as an indicator. The results are given in Table I. The acid was later utilized for after a time the reaction became alkaline. Gas was produced in abundance usually 60 to 75 per cent in d glucose, d fructose, d mannose, maltose and trehalose and was mostly carbon dioxide. On some of the sugars a pellicle occurred on the surface of the media; in others most of the growth took place at the bottom of the tube. The latter was true particularly of the sugars which were fermented with the production of acid and gas.

Cellulose and starch were not utilized. Filter paper moistened with dextrose peptone solution and inoculated with the organism was not softened. Starch was tested by growing the organism on starch agar and flooding with Gram's iodine. Submerged mycelia were produced abundantly from colonies on starch agar. In a few days the feathery mycelia could be seen projecting into the agar from the surface of the round, smooth, moist surface colony.

courtesy of two of the hospitals and of the Hudson County Board of Health, records were obtained from which some generalization can be made. These cases may be divided into groups as shown in Table I.

TABLE I

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Number of patients	9	7	5	1
Phage therapy begun after admission to hospital	First day	2-4 days	5-15 days	Control
Average time for decrease of stool frequency	4½ days	7 days	12 days	12 days
Average days of hospitalization	18	13	28	22

Group 1 (9 patients) received phage on the day of admission; four and two-thirds days elapsed before the stools decreased in number and eighteen days of hospitalization were required. Group 2 (7 patients) received phage between the second and fourth days after admission; stools decreased in number after seven days, and the patients remained in the hospital thirteen days on the average. Group 3 (5 patients) did not receive phage therapy until between the fifth and fifteenth day after admission, and these showed reduction in number of stools at twelve days as did the control case, and required the longest period of hospitalization (twenty-eight days).

Bacteriophage was administered in doses of 2.5 c.c. to 15 c.c. a day, the average dose being approximately 10 c.c. daily. In this group of 17 children and 5 adults, the children were more severely ill, but there were no fatalities. It was unfortunate that there was but one control case to report. However, it is evident that, in those patients receiving early treatment, the frequency of stools was lessened and the period of hospitalization was somewhat shortened.

During this epidemic we had the opportunity to observe several cases in this hospital under bacteriophage treatment. Dysentery bacilli (Flexner group) were isolated from the stools of these patients. The organisms were completely susceptible to lysis and a phage was made specific for these strains by serial passage. The preparation used in therapy for these hospital cases consisted of 50 per cent specific Flexner phage and 50 per cent of the stock phage. A detailed account of these cases follows:

CASE 1.—S. B., male, aged four years, one of a family of four children, was admitted to the hospital Aug. 10, 1934. He was severely ill, dehydrated, and had a temperature of 102° F., with a pulse rate of 120. During the preceding week his temperature had ranged between 101° and 103° F. He had been passing daily as many as fifteen light green stools containing blood and mucus, with vomiting several times a day. This child and his brothers had been playing with three neighborhood children who had previously had similar attacks of diarrhea with blood in their stools. On the day of admission and on each of the three days following, the patient was given infusions of glucose. On the first day he passed four yellowish green watery stools containing mucus. One of these specimens was found positive for dysentery bacilli (Flexner group) which were completely lysed by the bacteriophage. On the second day, bacteriophage therapy was begun. The patient received three 5 c.c. doses by mouth and a retention enema of 10 c.c. of phage diluted with 1 or 2 ounces of starch solution. Nineteen stools were passed on this day. Twenty-six hours after starting bacteriophage the stools became slightly more formed with less mucus and no blood. On the fourth day (August 13), the child passed 31 stools with less mucus than previously had been observed. The oral administration of bacteriophage was increased to four doses of 5 c.c. each. On the

Oozing from it was a thick pus. The lungs were hemorrhagic and there was a small yellowish abscess in one testicle. Direct smears made from the liver, omentum, spleen, kidney, adrenal, lung, and testicle showed the organism and cultures were obtained on Sabouraud's medium and dextrose tartaric medium from the liver, spleen, testicle, omentum, lung, and kidney.

A second rat injected intraperitoneally with a culture of the organism from this rat died in six days, much sooner than the first but otherwise presented much the same lesions. The mass found in the omentum was the same. There was no abscess in the testicle but there were a few in the liver. The organism was seen in direct smear from several of the organs and was also obtained in cultures.

The guinea pig died eleven days after subcutaneous inoculation. Subcutaneously at the site of injection a soft yellowish mass about 1.3 by 1 by 1 cm was found and a smaller mass in the omentum near the stomach. The lungs were edematous and the adrenals were enlarged. No yeastlike organisms were seen in direct smears made from these organs and stained with methylene blue but cultures of them were obtained from the lung and subcutaneous mass on Sabouraud's medium. This culture was injected subcutaneously into a second guinea pig, which died in nineteen days and presented almost the same findings. The anterior abdominal wall showed a crust covered hypertrophied lesion 2 cm in diameter from which a thick, creamy pus could be squeezed. This extended through the abdominal wall causing a small lesion in the peritoneum. Two slightly enlarged lymph glands were found near this lesion. Near the pylorus of the stomach a mass of omentum about 1 cm in diameter was found. In the center of this mass was a small grayish lesion about 1 mm in diameter. Smears made from the subcutaneous lesion showed the presence of the yeastlike organism, but none were found in the smear from the lesion in the omentum. No cultures were made.

The mouse did not die until thirty seven days after injection. A small subcutaneous abscess was found at the site of the injection. The inguinal glands were enlarged and the kidneys were edematous. A smear from the pus in the abscess revealed diplococci and the yeastlike organisms and smears from the gland, heart blood and kidney showed the yeastlike organism. Cultures made on Sabouraud's and dextrose tartaric acid media were negative for the yeastlike organism.

A suspension of the original culture from the patient was injected subcutaneously into a second mouse. This mouse died in twenty nine days. A thickened and reddened mass of the omentum was found near the stomach. No other evidence of infection was found. Smears made from the omentum showed the presence of the yeastlike organism, and cultures were secured on Sabouraud's and dextrose tartaric acid media.

*Histology of Lesions in Guinea Pig and White Rat.* Sections made from the lesion at the site of injection in one guinea pig showed large abscess formation, consisting of necrosis and diffuse infiltration of neutrophils, lymphocytes and monocytes adjoined by a rather loose proliferation of connective tissue consisting of rather faintly stained elongated nuclei and loosely woven fibrils. The scanty leucocytic reaction in this portion of the section consisted chiefly of lymphocytes and monocytes.

Sections made from the mass in the omentum of one rat showed a large area of necrosis accompanied by a diffuse leucocytic infiltration of neutrophils, lymphocytes and monocytes almost completely surrounded by a loose connective tissue proliferation similar to that seen in the guinea pig. This connective tissue was studded with numerous foreign body giant cells and showed a scanty infiltration of leucocytes, chiefly lymphocytes and monocytes.

*Classification.* Henrici<sup>2</sup> says "The classification of the fungi imperfecti is very unsatisfactory. Undoubtedly in a large proportion of them the imperfection lies in our knowledge of their life cycles." The clinical features of the case, the occurrence of subcutaneous tumor masses, the cutaneous and lung lesions are similar to the infection described by Gilchrist<sup>3</sup> as caused by *Blastomyces dermatitidis* or *Oidium dermatitidis*.

Culturally the organism did not resemble it. It did not produce a tough growth on solid media characteristic of *Oidium dermatitidis*, and it did not produce aerial mycelium. *Oidium dermatitidis* does not ferment any carbohydrates, this organism fermented several carbohydrates with the production of acid and gas.

Michelson,<sup>3</sup> however, reports two cases of systemic blastomycosis in which only yeast forms were obtained on culture.

stools containing blood and mucus. On the day of admission bacteriophage therapy was begun with two doses of 5 c.c. each by mouth. He passed three green and brown semisolid stools with a moderate amount of mucus. A stool cultured on this day was positive for dysentery bacilli. The next day bacteriophage was increased to five doses by mouth and one

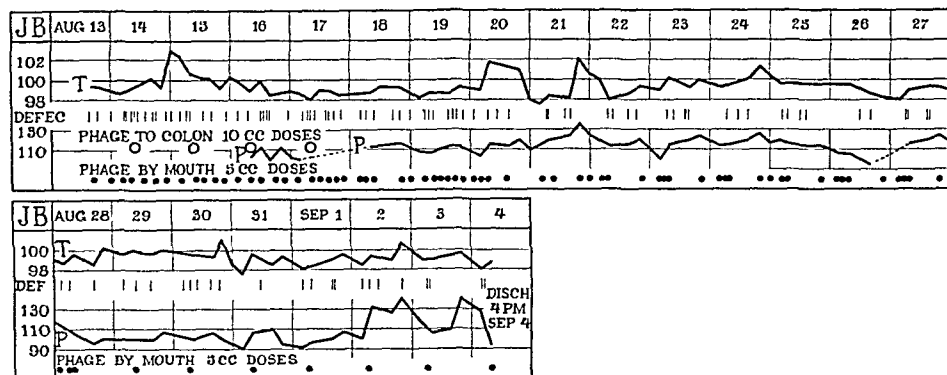


Chart 3.

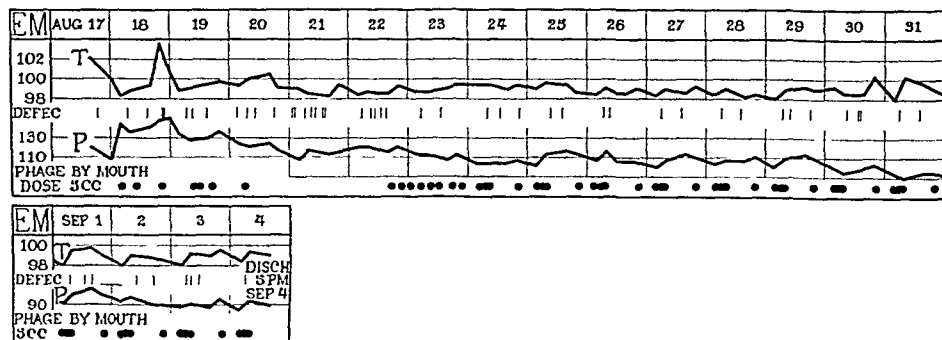


Chart 4.

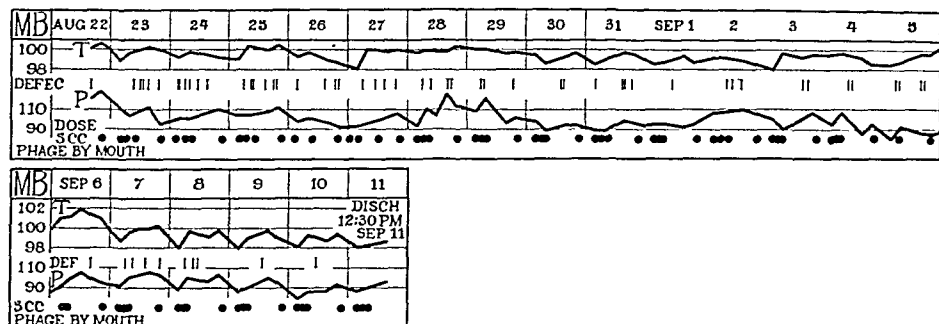


Chart 5.

10 c.c. enema was given. The patient passed ten stools, most of which contained blood. On the third day, he passed fewer stools and seemed much improved. On the tenth day, the stools were semisolid without blood. The patient was discharged September 4.

CASE 4.—E. M., male, aged twenty months, was admitted to the hospital on Aug. 17, 1934, with a temperature of 102° F. and a pulse of 120, fretful and severely ill. There was a

The nodular lesions of the face were differentiated from leprosy and *Tuberculosis cutis*. Nodular lesions of leprosy as a rule are easily diagnosed by the finding of numerous acid fast bacilli, many of which are characteristically arranged in globi. The failure to find these organisms at once should put the observer on guard for some other etiologic agent. To rule out *Tuberculosis cutis* it was necessary to find another etiologic agent as the finding of tubercle bacilli in these lesions is sometimes very difficult. The observing of the yeastlike organism in smear and the histologic picture ruled in this.

The subcutaneous tumors of the wrist and forearm could be diagnosed only after histologic section and culture with the finding of monilia.

The bone lesions of the fingers might well be looked upon as being caused by the same organism although no proof of this can be given. These lesions were closed except for one spot on one of the fingers and it did not seem justifiable to disturb such a lesion for a specimen. However, bone lesions have been reported as being caused by yeastlike organisms. The absence of anesthesia seemed to rule out leprosy with which the bone absorption could have been associated.

It is interesting to note the finding of foreign body giant cells in the nodular lesion on the patient's face and in the lesion produced in the omentum of the white rat.

#### SUMMARY

A case of monilia infection of the skin and lungs with the production of subcutaneous myxomatous tumors and possibly the involvement of bone, in a colored man, is presented.

Bacteriologic studies showed that the organism was a strain of monilia.

Experimental data prove the organism is pathogenic for rabbits, white rats, guinea pigs, and mice in the order named. Inflammatory tumor masses were produced in the omentum of white rats at the site of injection in guinea pigs and in the omentum of one mouse in which monilia were found to be present.

#### REFERENCES

- 1 Draper, A. A. Biology of *Oidium albicans*, With Special Reference to Mycelial Production, *J Infect Dis* 39: 261, 1926.
- 2 Hensley, J. D. Molds, Yeast and Actinomycetes, New York, 1930, John Wiley and Sons, Inc.
- 3 Michelson, I. D. Blastomycosis, Pathologic and Bacteriologic Study, *J A M A* 91: 1871, 1928.
- 4 Spring, D. Comparison of 7 Strains of Organisms Causing Blastomycosis in Man, *J Infect Dis* 44: 169, 1929.
- 5 Smith, L. W. Role of *Monilia psilosis* (Schfordi) in Experimental Sprue, Including Mycologic Observations on 21 Strains of Monilia, *J A M A* 83: 1549, 1924.
- 6 Rewbridge, A. G., Dodge, C. W., and Ayers, T. T. Case of Meningitis Due to *Endomyces capsulatus* (New Species), *Am J Path* 5: 349, 1929.
- 7 Wilson, S. J., Hulsey, S., and Weidman, F. D. Chromoblastomycosis in Texas, *Arch Dermat & Syph* 27: 107, 1933.
- 8 Connor, C. L. Monilia From Osteomyelitis, *J Infect Dis* 43: 108, 1928.
- 9 Benham, R. W. Fungi of Blastomycosis and Coccidioidal Granuloma, *Arch Dermat & Syph* 30: 385, 1934.
- 10 Hiss and Zimmsen. Textbook of Bacteriology, New York, 1927, D. Appleton Century Co., Inc.
- 11 Castellani, A. Fungi and Fungous Diseases, *Arch Dermat & Syph* 16: 383, 1927.
- 12 Smith, L. W., and Sano, M. E. Moniliasis With Meningeal Involvement, *J Infect Dis* 53: 187, 1933.

## REFERENCES

1. D'Herelle, F.: *Le bactériophage: son rôle dans l'immunité*. Paris, Masson et Cie, 1921, 277 pp., Monographie de l'Inst. Pasteur.
2. Cruz, J. da Costa: Bacteriophage in Therapeutics, *Brazil-med.* 1: 298, 1923; *Abst. J. A. M. A.* 81: 698, 1923.
3. Cruz, J. da Costa: Bacteriophage in Treatment of Dysentery, *Compt. rend. Soc. de biol.* 91: 845, 1924; *Abst. J. A. M. A.* 83: 1542, 1924.
4. Spence, R. C., and McKinley, E. B.: The Therapeutic Value of Bacteriophage in Treatment of Bacillary Dysentery, *South. M. J.* 17: 536, 1924.
5. Compton, A.: Anti-Dysentery Bacteriophage in the Treatment of Bacillary Dysentery, a Record of 66 Cases Treated, with Inferences, *Lancet* 2: 273, 1929.
6. Choudhury, B. K. P., and Morison, J.: The Spread of Dysentery in a Khasi Village and Its Treatment with Bacteriophage, *Indian M. Gaz.* 64: 66, 1929.
7. Davison, W. C.: The Bacteriolysant Therapy of Bacillary Dysentery in Children, *Am. J. Dis. Child.* 23: 531, 1922.
8. Taylor, J., Greval, S. D. S., and Thant, U.: Bacteriophage in Bacillary Dysentery and Cholera, *Indian J. M. Research* 18: 117, 1930.
9. Riding, D.: Acute Bacillary Dysentery in Khartoum Province, Sudan, with Special Reference to Bacteriophage Treatment: Bacteriological Investigation, *J. Hyg.* 30: 387, 1930.
10. McCay, F. H.: Treatment of Bacillary Dysentery by Bacteriophage, *Indian M. Gaz.* 67: 666, 1922.
11. Johnston, M. M., Ebbs, J. H., and Kaake, M. J.: Bacteriophage Therapy in Acute Intestinal Infection (Summer Diarrhea), *Canad. Pub. Health J.* 24: 443, 1933.
12. Kessel, J. F., and Rose, E. J.: Bacteriophage Therapy in Bacillary Dysentery of Flexner Type, *Ann. Int. Med.* 6: 1193, 1933.
13. Felsen, Joseph, Rundlett, Emily V., Sullivan, James, and Gorenberg, Harold: Atypical Flexner Dysentery, *J. A. M. A.* 103: 1055, 1934.

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 THE DIURETIC ACTION OF GLUCOPHYLLINE\*
 

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GLUCOPHYLLINE, one of the newer diuretics of the xanthine series, is a double salt representing a mixture of theophylline and methyl glucamine, having a xanthine content of 38.7 per cent. It differs from aminophylline in that the latter represents a mixture of theophylline and ethylene diamine, having a xanthine content of 59 per cent.

In determining the diuretic activity of this newer preparation, we have carried on a series of experiments on laboratory animals and human volunteer subjects, the results of which are presented in this report.

## LABORATORY EXPERIMENTS

Dogs were used for quantitative determination of the diuretic action of glucophylline. Both ureters were cannulated and the urine flow was measured for a specific time interval in each experiment. In most instances the blood pressure and respiration curves were also taken at the same time. In the first four experiments, the animals were anesthetized with dialurethane or nembutal, and the diuretic action of glucophylline was tested against that of citrated caffeine, aminophylline, and salyrgan. In the last ten experiments, to reduce complications, pernocton was used exclusively to produce anesthesia, and the

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fact that therapy was started too late in the course of the disease Taylor, Gieval and Thant,<sup>8</sup> in 1930, reported bacteriophage treatment of 14 Shiga and 6 Flexner dysentery cases in which only a short interval elapsed between onset of the disease and treatment. Two cubic centimeters of polyvalent phage were given three times a day. In the treated Shiga group there was a mortality of 12 per cent and in the control group 14 per cent. Out of 6 patients in the control and treated Flexner groups there was one death in each series. Riding,<sup>9</sup> in 1930, reported 60 cases. 30 patients treated with phage by mouth and 30 untreated. Six types of dysentery bacilli were isolated, and all but 12 strains were susceptible to the phage used. No clinical benefit was noted in the phage treated patients. In 1932 McCay<sup>10</sup> reported treating a large number of patients in Calcutta with bacteriophage. Dysentery bacilli of the Flexner type were isolated in most of the cases. Twenty five cubic centimeter doses were given twice a day, and in more severe cases this dose was increased. The deaths in the phage treated group were 5.4 per cent in the control group 10.8 per cent. The average time of hospitalization in the treated group was more prolonged than in the control group, although 30 per cent of the phage treated patients recovered rapidly. In spite of rather conflicting evidence, McCay concludes that this form of therapy was not generally beneficial. In 1933, Johnston, Ebbs and Kaake<sup>11</sup> found that bacteriophage did not affect the clinical course of the dysentery. Of the 70 patients considered all infants under two years, pathogenic organisms were isolated from the stools of 73 per cent. Only 17 strains of the 94 tested in vitro were lysed by the bacteriophage employed. The dosage used was one ounce every hour. Kessel and Rose,<sup>12</sup> in 1933, reported 68 cases, half of which were treated, the remainder serving as controls. Ninety per cent of the Flexner strains were found susceptible to the phage employed. The dosage was 3 cc to 5 cc of phage by mouth every twelve hours. There were 3 deaths in the control group and 4 deaths in the treated group. The period of hospitalization was somewhat shorter in the treated group.

#### EXPERIMENTAL

During the summer of 1934, an epidemic\* of bacillary dysentery developed in Jersey City, New Jersey. Stool specimens from several of the patients were sent to our laboratory for diagnosis and bacteriophage susceptibility tests. From these specimens dysentery bacilli (Flexner group) were isolated. This organism was found to be completely susceptible, holding its lysis for twenty four hours when treated with a mixture of approximately forty races of bacteriophage active against the colon bacillus.

With the dysentery strains isolated from these specimens we prepared bacteriophage in beef infusion broth which was used extensively in Jersey City and the surrounding territory.

Although there were, during the epidemic, approximately 200 cases of bacillary dysentery treated with bacteriophage supplied by this laboratory, difficulty was encountered in evaluating the results due to the fact that in the majority of cases the records were not made available to us. Through the

\*A preliminary report upon this epidemic has been made by Felsen, Rundlett, Sullivan and Gorenberg.<sup>13</sup>

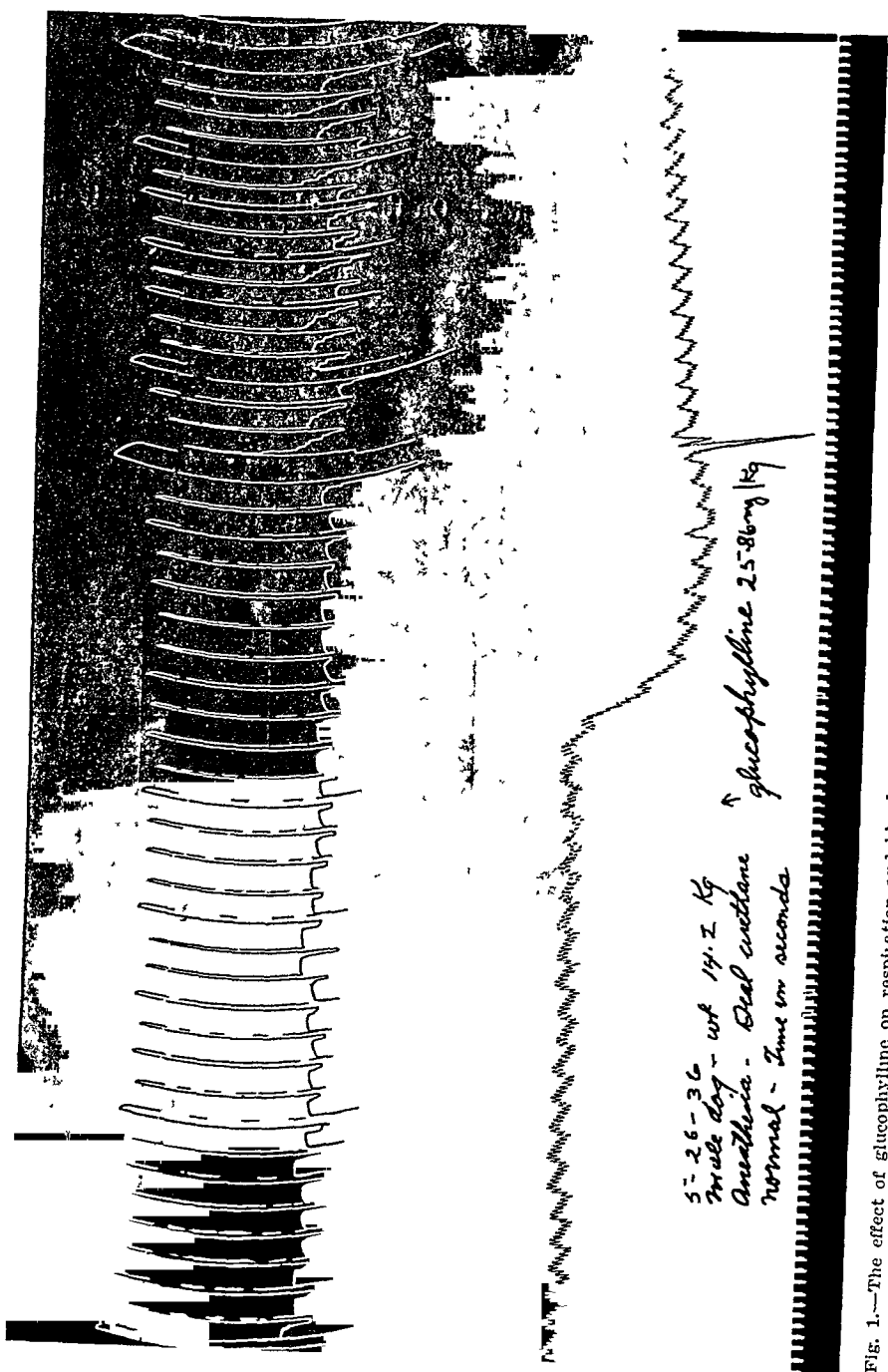


Fig. 1.—The effect of glucophyllyne on respiration and blood pressure; upper tracing, respiration; middle tracing, blood pressure, lower tracing, time in seconds.

fifth day the stools appeared more formed, bacteriophage was increased to five doses. On the seventh day the patient seemed brighter. On the ninth day the bacteriophage enemas were discontinued because of the inability of the patient to retain them. Convalescence was uneventful and the child was discharged from the hospital September 4.

CASE 2—A B, male, aged six years, was admitted to the hospital Aug 11, 1934, eighteen hours after his brother S B. The child presented less toxic symptoms than his brother and his temperature was lower at the time of admission. During the preceding twelve hours, the patient had had a temperature of 103° F and had passed three loose, green, watery stools containing large amounts of mucus and blood. On the day of admission his temperature was 103.6° F., and pulse 112. A stool specimen sent to the laboratory was positive for

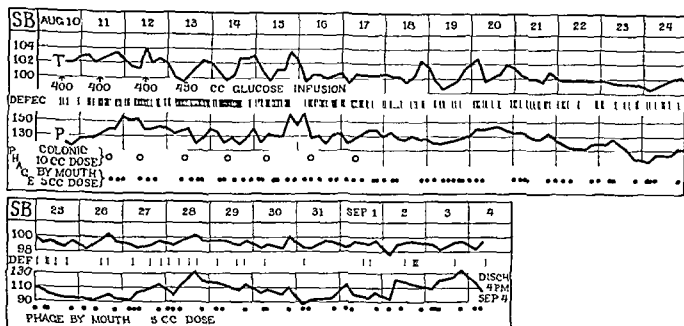


Chart 1

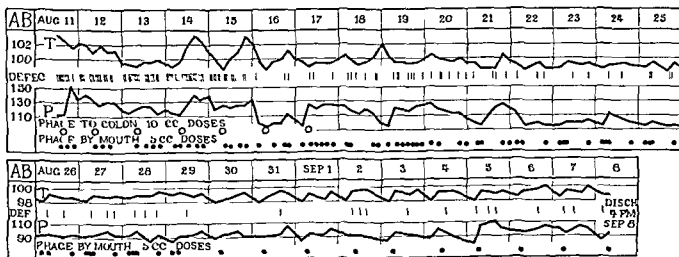


Chart 2

dysentery bacilli. Bacteriophage therapy was begun at once with three doses of 5 c c each by mouth and a starch enema containing 10 c c of phage. The child passed twelve stools. On the second day, a stool passed in the morning was semiformed and contained less blood. On the third day his temperature varied between 99° and 100° F and the child was more active. The stools were semisolid with less blood and mucus. Bacteriophage was increased to four doses of 5 c c each by mouth. On the fifth day, the stools were greatly decreased in number. The child appeared less irritable. On the thirteenth day (August 23), the stools were formed and there was no blood present. The patient was discharged September 8.

CASE 3—J B, male, aged seventeen months, brother of the preceding patients, was admitted to the hospital August 13. On the previous day he had passed three green watery

acetate (theocin), theobromine sodium salicylate (diuretin), theophylline ethylene diamine (aminophylline), and caffeine alkaloid. In addition, a few series were run with methyl glucamine to determine the rôle played by this salt in the mixture. In no case was a subject informed what the drug was at the time of administration and, to further negative the psychic factor a series was run with bicarbonate of soda as the test drug. Our procedure was as follows: An average volume output of urine voided on six consecutive days was taken as the standard for comparative purposes. At 9:00 A.M. the urine was voided and discarded. All other samples for the day were kept, the last being at 5:00 P.M. This served as control. On test days the drug was given by mouth immediately after the 9:00 A.M. voiding and repeated at 12:00 M. Since it was practically impossible to secure a twenty-four-hour specimen, the duration

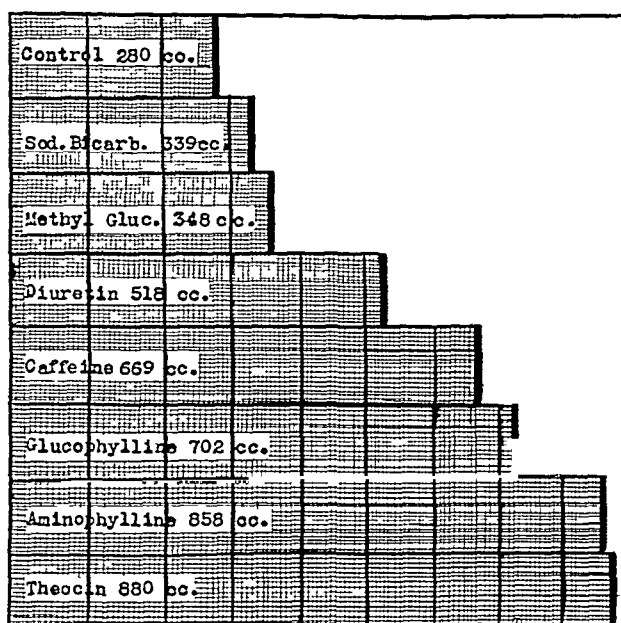


Fig 3 —Composite averages on nine human subjects

of action was determined by routine collections the following day. No restrictions as to diet or fluid intake were imposed, but to compensate for incidental variations more than one trial was made with each of the drugs and several with glucophylline. Routine urinalyses showed, in some, variation in the specific gravity and reaction and, in all, the constant absence of albumin and reducing substances. A record was kept on the volume output in each case. The dose of each compound was computed on the basis of its xanthine content, using caffeine as unity and bringing up the others to relative equivalency. Seven days were allowed to intervene between any two trials. Table II gives details on individual results.

Although this study is but roughly quantitative, certain definite deductions can be drawn from the figures presented in this table. Taking each of the nine subjects separately, glucophylline comes among the first three most effective diuretics, the other two being theocin and aminophylline, all belonging

history of vomiting and diarrhea for five days before admission. Green watery stools with blood and mucus appeared the day preceding admission. The following day three 5 cc doses of bacteriophage were given by mouth. In the evening the temperature was 103.6° F and the pulse 140. Four stools were passed this day, one of which was cultured and found positive for dysentery bacilli. The temperature decreased to 99° F on the third day and he appeared less fretful. On the eighth day the chill passed, three brown semisolid stools. He was discharged September 4.

CASE 5—M. B., female, aged three years, was admitted to the hospital Aug. 22, 1934. During the six days preceding admission the patient had passed daily from eight to ten loose, greenish brown stools which contained mucus and blood. For two days prior to admission, the child's temperature reached 102° F. On the day of admission the temperature was 101° F and pulse 120. One dose of 5 cc of bacteriophage was given. A stool specimen was positive for dysentery bacilli. The following day bacteriophage was increased to four doses of 5 cc each by mouth. On this day five watery brown stools containing mucus and blood were passed. On August 26, the fifth day, four stools were passed which were free from blood although some mucus was still present. On the eleventh day, only one stool was passed and that appeared normal. The patient was discharged September 11.

#### DISCUSSION

The value of bacteriophage as a therapeutic agent in bacillary dysentery has not been fully determined. The future use of such therapy may be guided by an analysis of previous work, with due regard to the causes of success and failure. Coordination of the laboratory and the bedside will be required for a just appraisal of this newer form of therapy.

Although there is no standard dosage for the treatment of bacillary dysentery, it is probable that the bacteriophage should be given every day for a considerable period. We would stress the importance of starting bacteriophage treatment as early as possible after onset of the disease and employing larger doses than were used by the earlier workers.

For the successful use of phage in dysentery it is important to determine the type of invading organism and to make *in vitro* tests for susceptibility of the strain to lysis. We prefer to use a strain specific phage which has been adapted to the patient's strain of dysentery bacilli, by serial passage. We believe this kind of phage to be more efficacious than stock preparations.

#### SUMMARY

- 1 Detailed observations are presented upon five cases of bacillary dysentery in which specific bacteriophage was used in therapy.
- 2 A larger number of patients supplied with phage from our laboratory during a mild epidemic of dysentery in a neighboring community provide a group from which general deductions may be drawn.
- 3 It seems advantageous to institute bacteriophage therapy as soon as possible after onset of the disease and to persist in its use.
- 4 The invading organism should be isolated and a strain specific phage prepared for use without delay.

*Acknowledgments*—We wish to express our appreciation of the helpful cooperation of the personnel of the Board of Health and Vital Statistics of Hudson County, New Jersey, Jersey City, New Jersey, of St. Mary Hospital, Hoboken, New Jersey and of St. Francis Hospital, Jersey City, New Jersey. We also desire to make grateful acknowledgment to Dr. Roger H. Dennett, deceased, on whose service the five hospitalized patients were treated.

to the theophylline group. This is in good agreement with the general observation that the theophylline group is more powerfully diuretic than the caffeine or the theobromine group. A determination of the diuretic effects on the second day showed that glucophylline has the longest duration of action. This is probably due to the methyl glucamine fraction for our protocols show this substance to be more active on the second even than on the first day. In chronic conditions, glucophylline possesses this advantage over aminophylline especially, which exhibits what appears to be a compensatory oliguria on the second day. In almost all instances theocin and glucophylline produced a change toward alkalinity in the reaction of the urine. This stands out in striking contrast to the other diuretics under investigation which almost uniformly produced an acid or neutral reaction. There is close correlation also in composite average figures on the diuretic efficiency of the various substances. Reference to the composite averages in Fig. 3 shows that glucophylline stands third in the series.

#### DISCUSSION

In both anesthetized dogs and normal human beings, glucophylline acted as a good diuretic agent. In our experiments, it exhibited an action superior to caffeine but similar to the other salts of theophylline. The only observable difference noted with respect to theocin and aminophylline was its longer latency. Its slightly reduced efficiency would be clinically inconsequential. It should be of interest to determine whether glucophylline exerts any untoward effects on the liver or kidney. Accordingly a series of chronic animal experiments are in progress with the view of studying these organs and other viscera histologically after a reasonably extended period of frequent medication.

#### SUMMARY

The results obtained on laboratory and clinical investigations of the diuretic action of glucophylline, the double salt of theophylline and methyl glucamine are presented.

The laboratory studies were made on dogs.

It was found that the average hourly urine excretion under glucophylline amounted to 9 c.c.; that under aminophylline to 7.2 c.c.

The clinical studies were made on seven sophomore medical students and two instructors.

In each individual case, glucophylline compared favorably with aminophylline and theocin and was superior to caffeine and diuretin.

In the composite averages of the nine subjects, glucophylline ranked third in the list of diuretics employed for comparative investigation. The diuretic action of theocin was 880 c.c., aminophylline 858 c.c., glucophylline 702 c.c., caffeine 669 c.c., and diuretin 518 c.c.

Glucophylline has a longer duration of diuretic action than aminophylline. This is probably due to the methyl glucamine fraction, which as a diuretic is more active on the second than the first day.

Appropriate tables and figures are presented.

We wish to thank the following pharmaceutical houses for supplying the drugs used in this work: The Abbott Laboratories for glucophylline and methyl glucamine, the H. E. Dubin Laboratories, G. D. Searle & Co., for aminophylline, Hoffmann-La Roche, Inc., for diuretin, and the Winthrop Chemical Co., for theocin.

diuretic action of glucophylline was tested against that of aminophylline only. In order to maintain a fairly constant condition of body fluid, slow hypodermoclysis or rectal drip with normal saline was given throughout the course of each experiment. No new drug was given until the urinary output under the preceding test drug had returned to the normal value for a given experiment.

The records show that in adequate doses the respiratory rate and volume were increased by all the diuretic agents. Of these, the mercurial diuretic salyrgan had the most pronounced effect. On the blood pressure an early but rather transitory fall was registered by all. Increase in urine excretion seemed to bear relation to restoration of the blood pressure to the preexisting level (or above), for in no instance did the optimal excretion occur until the blood pressure regained its normal level. There was observable, therefore, a definite latency with each compound. It was impossible, however, to construct comparative curves of latency because of the lack of uniformity of response on the part of the individual animals to the compounds tested.

An analysis of the results presented in Table I shows that glucophylline compares favorably in its diuretic efficiency with aminophylline. In the ten

TABLE I

DOGS COMPARATIVE DIURETIC ACTION OF COMPOUNDS TESTED

DOG	WEIGHT KG	ANESTHETIC	DRUG	DOSE MG /KG	URINE EXCRETED
1	F 65	Nembutal	Aminophylline	10.0	25
			Glucophylline	10.0	35
			Salyrgan	10.0	35
2	M 15.0	Nembutal	Citrated caffeine	17.0	33
			Aminophylline	13.0	35
			Glucophylline	17.0	30
			Salyrgan	20.0	10
3	M 13.5	Dialurethane	Citrated caffeine	10.0	23
			Aminophylline	10.0	10
			Glucophylline	10.0	30
4	M 12.5	Dialurethane	Citrated caffeine	10.0	33
			Aminophylline	8.0	33
			Glucophylline	10.0	50
			Salyrgan	20.0	40
5	F 4.6	Pernocton	Aminophylline	14.28	30
			Glucophylline	25.86	35
6	M 14.2	Pernocton	Glucophylline	25.86	996
			Aminophylline	14.28	192
7	F 8.2	Pernocton	Glucophylline	25.86	240
			Aminophylline	14.28	168
8	F 8.4	Pernocton	Aminophylline	14.28	744
			Glucophylline	25.86	192
9	M 13.4	Pernocton	Glucophylline	25.86	90
			Aminophylline	14.28	576
10	M 13.6	Pernocton	Glucophylline	25.86	96
			Aminophylline	14.28	10
11	M 5.7	Pernocton	Aminophylline	14.28	924
			Glucophylline	25.86	750
12	F 5.6	Pernocton	Aminophylline	14.28	750
			Glucophylline	25.86	150
13	M 13.4	Pernocton	Aminophylline	14.28	1206
			Glucophylline	25.86	90
14	M 13.6	Pernocton	Aminophylline	14.28	246
			Glucophylline	25.86	096

In a recent study reported by Leverton and Roberts,<sup>7</sup> they found that the standard daily deviation for women was 0.9 gm. of hemoglobin per 100 c.c., even in subjects under controlled conditions.

Rabinovitch,<sup>8</sup> in 1923, studied the hourly hemoglobin changes in 20 normal male individuals from 8 A.M. to 6 P.M. As much as 26 per cent variation was found in 2 cases, in 4 cases the variation ranged from 15 to 20 per cent, and in 6 cases from 10 to 15 per cent. Mills<sup>9</sup> investigated the hourly changes which occurred in persons with secondary anemia and found only a 4 per cent fluctuation. He concluded that the hourly irregularities were less pronounced in anemic persons than in normal healthy persons. On the contrary, Dreyer and others<sup>6</sup> found a more marked daily deviation in persons with a low hemoglobin level.

It does seem to be generally conceded that there are both daily and hourly fluctuations in hemoglobin and that these are more pronounced in women than in men. Ingersoll<sup>10</sup> found a 1.54 gm. average difference in women as compared to 1.10 gm. in men. Whether or not the variation in females can be attributed, at least, partially to menstrual changes causing an instability of the hematopoietic system and increased physiologic demands for iron is a subject of controversy. Smith, 1936,<sup>11</sup> studied 16 menstrual periods and concluded that increases and decreases in hemoglobin during the menstrual period were no different than during the intermenstrual period. Leverton and Roberts<sup>7</sup> summarized their data, after making an extensive study, by stating that the effect of menstruation was not definite or consistent. Reich and Green<sup>12</sup> pointed out that there was no postmenstrual reticulocyte peak, which would be evidence that there is no definite blood regeneration following menstruation. In contradiction to these findings, Ashby<sup>13</sup> reported that the blood-destroying activity in women was coincident with menstruation. Rowe and Guagenty<sup>14</sup> found that there was a downward trend during the time of menstrual flow and a gradual recovery after its termination. However, the average change was not great.

Barer and associates<sup>15</sup> analyzed the normal menstrual loss for 50 healthy women, nineteen to forty-three years of age, who presented no known menstrual disorders. They found that 3.84 to 78.4 mg. of iron was lost per period, representing 1.146 to 23.403 gm. of hemoglobin. This loss was equivalent to 9.39 to 207.28 c.c. of blood, calculated from the individual's own hemoglobin content in grams per 100 c.c. Patients with hypochromic anemia, even though they considered their menstrual periods normal, showed larger losses. According to their calculations, a loss of 78.4 mg. of iron each menstrual period would require a daily iron retention of about 2.8 mg. to replace that lost.

#### EXPERIMENTATION

Seven healthy college women, twenty to twenty-seven years of age, served as subjects for this investigation. Five of them, Cases 1 to 5, had had hemoglobins taken at intervals before the study was begun and were known to be nonanemic individuals. The sixth woman, Case 6, had shown a rather low hemoglobin level for a short period about six months previous to this time. She was given iron therapy, 25 mg. daily in the form of ferric pyrophosphate,

experiments under pernocton anesthesia in which the doses of these two compounds represented an equivalence of xanthine content, the average hourly excretion was 9 c.c. for glucophylline as against 72 c.c. for aminophylline.

Typical tracings showing the effects of glucophylline and aminophylline on blood pressure and respiration are presented in Figs. 1 and 2



Fig. 2—The effect of glucophylline on respiration and blood pressure. upper tracing, respiration middle tracing, blood pressure lower tracing, time in seconds

#### CLINICAL EXPERIMENTS

Seven male medical students and two of us volunteered to serve as subjects for this group of experiments. A renal function test and a complete urinalysis were done on each subject as a preliminary step. All subjects were found to be normal as to functional capacity and their urines were negative for albumin and redning substances. Four xanthine diuretics were employed for purposes of critical comparison with glucophylline: Theophylline sodium

Chart 1 shows a typical individual graph for one of the 5 subjects who had no history of anemia and also gives the records of the 2 women who were receiving treatment for slight hypochromic anemia. It will be noticed that the patient in Case 6 has a hemoglobin within the normal range, averaging 14.13 gm., throughout the study; while the patient in Case 7 shows a hemoglobin level lower than the others, averaging 12.19 gm., although she was receiving treatment. The daily variations are significant in all cases. They do not appear to be any more or less marked in Case 7 than in any of the others.

Chart 2 summarizes the average hemoglobin values computed from 214 determinations on the 5 women whose hemoglobins were consistently non-anemic. The range is from 13 to 15 gm. of hemoglobin per 100 c.c., and the average is 14.29 gm. Although this figure is based on a study of only 5 individuals, it probably represents a true average of the standard hemoglobin value for women of this age, since determinations were made almost daily for fifty-two consecutive days on subjects who were known to be normal healthy women. Certainly, a single hemoglobin determination is not likely to be

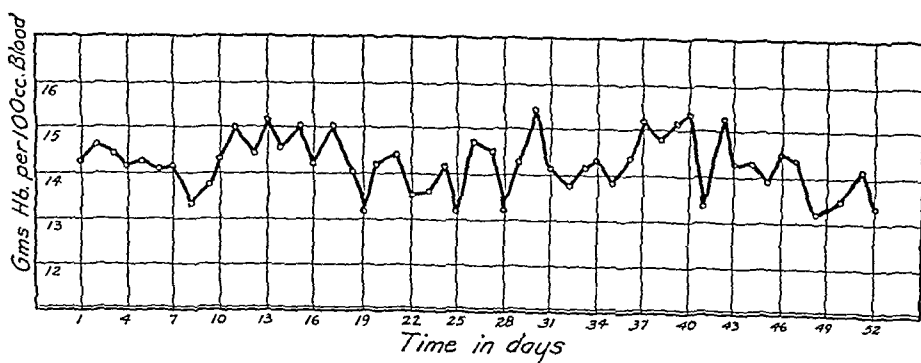


Chart 2.—Average hemoglobin curve constructed from 214 determinations on 5 normal cases.

indicative of the average level. Since determinations are often made by methods far less accurate than the colorimetric one used in this study, there is striking evidence as to the limitations of making a clinical diagnosis on the basis of one or two readings.

Hourly determinations were made on the patients in Cases 1, 3, and 6 to study the changes occurring within the day. The blood samples were taken from 7 A.M. through 10 P.M., and the colorimetric readings were made by daylight the following morning. Two one-day periods were taken as probably being exemplary of the hourly changes which usually occur. One day was during the intermenstrual period, approximately midway between menstrual periods, and the other was the second day of menstruation. This is the day of greatest hemorrhage for most healthy women.<sup>16</sup> Each subject had eight hours' rest the night before the hourly readings were made, and a careful record of food consumption was kept. The days chosen were typical days of activity.

In Chart 3, the two daily records for each of the 3 women are presented. In all cases, the irregularities are appreciable. The variations on the second

TABLE II  
HUMAN SUBJECTS COMPARATIVE DIURETIC ACTION OF COMPOUNDS TESTED

SUBJECTS COMPOSITE NORMAL VOL UMF, cc	1		2		3		4		5		6		7		8		9	
	VOL	%	VOL	%	VOL	%	VOL	%	VOL	%	VOL	%	VOL	%	VOL	%	VOL	%
TEST DIETS																		
Glucophylline	927	421	643	70.0	920	280	525	210	709	225	578	192	477	241	930	245	1560	578
Theocin	1155	725	645.0	67.1	1365	413	698	279	49	283	575	205	607	311	945	49	1240	475
Aminophylline	1410	641	437.5	15.0	1205	365	528	211	920	260	69	248	785	403	1200	313	775	287
Diuretin	908	412	492.5	40.0	438	133	325	170	763	244	311	227	338	173	480	126	410	152
Caffeine	870	395	640.0	168.5	455	138	630	260	908	288	615	219	605	310	540	112	685	254
Methyl glucamine	375	170	327.0	86.0	305	97	230	88	360	214	268	131	262	134	455	120	890	320
Sodium bicarbonate	450	204	290.0	76.0	510	155	270	108	290	92	240	86	320	164	-	-	-	-

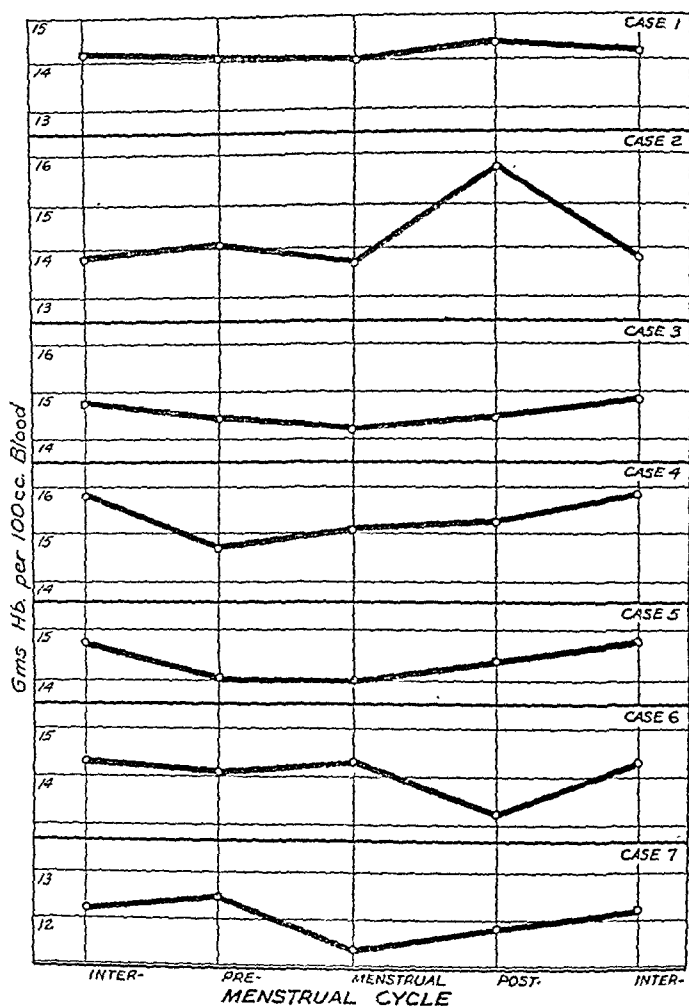


Chart 4.—Individual curves for 7 cases showing average hemoglobin values during the 4 phases of the menstrual cycle.

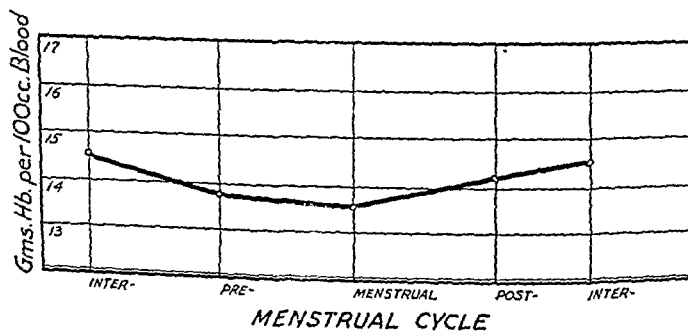


Chart 5.—Average curve constructed from 7 cases showing hemoglobin change with menstruation.

## HEMOGLOBIN STUDIES ON COLLEGE WOMEN WITH SPECIAL REFERENCE TO THE EFFECT OF MENSTRUATION\*

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THIS study of the hemoglobin content of the blood of seven college women, ranging from twenty to twenty seven years of age, was undertaken to determine the daily and hourly fluctuations which may occur. Hemoglobin determinations were made for a period of fifty two consecutive days, covering approximately two menstrual cycles. One complete cycle represented for each woman was divided into the four phases: premenstrual, menstrual, post menstrual, and intermenstrual, and an effort was made to correlate hemoglobin changes with these various phases. Hourly hemoglobin determinations were made on three of the subjects, once during the intermenstrual period and also on the second day of menstruation, to study the changes occurring within the day.

### LITERATURE

A survey of the literature indicates that this subject is one which has not been studied extensively but has received increased attention during the past few years. Williamson,<sup>1</sup> in 1916 investigated the influence of age and sex on hemoglobin and reported that from the ages of sixteen to fifty five years the variations were slight. During that period of life, the values for women were found to be appreciably lower than for men: 15.53 gm per 100 cc as compared to 16.92 gm. More recent work has shown that this standard for women, particularly, is probably too high. In 1927, Lippincott<sup>2</sup> concluded that 13.7 gm was the average hemoglobin for females. Murphy and others<sup>3</sup> reported an average of 13.9 gm and Helmer and Emerson<sup>4</sup> gave a similar figure of 13.68 gm. Sachs and coworkers have suggested a slightly lower value of 12.96 gm for women in comparison to 14.96 gm for men. From these data, a range of 13 to 14 gm of hemoglobin per 100 cc of blood would seem to be normal for women.

That diurnal variations in the hemoglobin content of the blood are marked has been realized but not given serious attention. In 1920 Dwyer and associates<sup>5</sup> noted that these fluctuations were as high as 30 per cent and that a change of 10 per cent was a common occurrence. They gave as possible causes of these fluctuations: blood pressure, rate and volume of respiration, variations in pulse rate, and possibly fluid absorption and kidney excretion.

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11. Smith, C.: Daily Erythrocyte Counts in Menstrual and Inter-Menstrual Periods, *Am. J. Physiol.* 114: 452, 1936.
12. Reich, C., and Green, D.: Red Cell Regeneration During the Menstrual Cycle, *Arch. Int. Med.* 49: 534, 1932.
13. Ashby, W.: Study of Transfused Blood, *J. Exper. Med.* 34: 127, 1921.
14. Rowe, A. W., and Guagenty, M. C.: A Note on the Menstrual Influence on Blood Morphology, *J. LAB. & CLIN. MED.* 20: 253, 1934.
15. Barer, A. P., Fowler, W. M., and Baldrige, C. W.: Blood Loss During Normal Menstruation, *Proc. Soc. Exper. Biol. & Med.* 32: 1458, 1935.
16. Novak, E.: Menstruation and Its Disorders, New York, 1931, D. Appleton and Co.
17. Dodds, G. S.: Essentials of Human Embryology, New York, 1929, John Wiley and Sons.

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### THE KAHN TEST IN MALARIA\*

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THE first communications<sup>20</sup> regarding a positive Wassermann reaction in malaria date back to the years 1908 and 1909 when Michaelis and Lesser, Much and Eichelberg and others raised the question. These reports together with his own observations led A. von Wassermann, the originator of the test, to despatch G. Meyer to Italy for the purpose of studying the matter. Meyer reported that 80 per cent of all cases of malaria with parasites in the blood showed a positive Wassermann reaction. Since then most observers have confirmed these findings, though in varying degrees.

Craig, in Hagen's textbook<sup>1</sup> says: "Of the few conditions in which a positive Wassermann reaction sometimes occurs are . . . malarial fevers, during the febrile stage." During the past twenty years a large number of observers have reported their experience with the Wassermann test in malarial infection. Without attempting to exhaust the list, one may mention St. John<sup>2</sup> who found a positive Wassermann reaction in 4.9 per cent of his malarial patients; Curth,<sup>3</sup> in 12.5 per cent; DeHaan,<sup>4</sup> in 19 per cent; Mayr,<sup>5</sup> in 20 per cent; Schilling,<sup>6</sup> in 33 per cent; Hirsch,<sup>7</sup> in 36 per cent; Hebewerth and Kop,<sup>8</sup> in 50 per cent; Fischer and Günsberger,<sup>9</sup> in 75 per cent; Nagell,<sup>10</sup> in 84 per cent. All of them excluded patients that gave a history suggestive of syphilis and state that the reaction became negative in a period varying from several days to many weeks after defervescence.

The great variation in the frequency of these false positives as reported by the various observers can only be explained by differences in technic and justifies at least the suspicion that Kolmer<sup>11</sup> may be right when he asserts that the so-called false positives in malaria are always due to faulty technic. Using his own modification of the Wassermann test, he found that in 26 unselected cases of tertian and estivo-autumnal malaria none gave a positive Wassermann reaction. In another series of malarial serums, sent him from Panama and Texas, all were negative to the Wassermann test except 2 in whom the possibility of syphilis could not be excluded. Busineo and Foltz<sup>12</sup> found a positive Wassermann reaction in 5 out of 49 cases of malaria but state that in all 5

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for three weeks, and her hemoglobin was raised from 12.40 to 13.89 gm per 100 cc of blood. At the time this experimental work was undertaken, the same treatment was started again although her hemoglobin was 13.61 gm. She continued taking iron for the fifty two days during this investigation. The seventh subject, Case 7, had been a known diabetic for thirteen years. She was taking 30 units of insulin daily and was receiving a carefully weighed adequate diet. She was in good health but had an average hemoglobin of approximately 12 gm per 100 cc of blood, so was given iron and copper throughout the fifty two day period. Twenty five milligrams of iron in the form of ferric pyrophosphate and 1 mg of copper from copper sulphate were administered daily. She had been receiving the same therapy at intervals

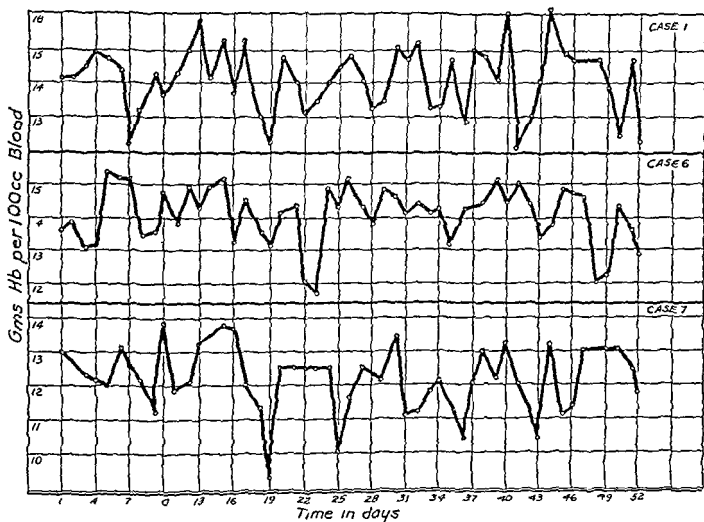


Chart 1—Typical individual curves showing daily variations in the hemoglobin content of the blood. Case 1 normal woman. Case 6 receiving 25 mg Fe daily. Case 7 a diabetic receiving 25 mg Fe and 1 mg Cu daily.

previous to this time. All of the women were home economics students at this university, had studied dietetics, and were familiar with the requirements of an adequate diet. Their nutrition was good.

Hemoglobin determinations were made daily with few exceptions for a period of fifty two consecutive days in order to observe the variations which occur. Cutaneous blood samples were taken from the fingertips. Each person's hemoglobin was taken at a corresponding time each day, usually from 10 A.M. to 1:30 P.M. The Newcomer method was used to determine the hemoglobin, the sample being compared with a carefully standardized disc in a Bausch and Lomb colorimeter.

interesting observation was that, whereas only 2 of these showed a positive Kahn and a negative Wassermann reaction, 6 showed a negative Kahn reaction and a positive Wassermann reaction. In our series, no patient with a positive Wassermann reaction failed to give a positive Kahn test.

These findings leave one in something of an impasse. On the one hand, a number of careful observers working with a large material have failed to find any definite relation between malaria and a positive Wassermann reaction; on the other, a still larger number of clinicians have seen, in a considerable percentage of malarial cases, a Wassermann reaction, strongly positive during the attack, becoming permanently negative after an interval of from six days to six weeks after defervescence. These cases can hardly all have been syphilitic, nor is it easy to explain the findings as due to technical error. The divergence of the results still awaits explanation.

Several of the clinicians<sup>9, 10, 17</sup> who have obtained positive Wassermann reactions in cases of malaria, report a similar experience with the flocculation tests, especially those of Sachs-Georgi and Meinicke. The Kahn test also has been found to give false positives in malaria. Curth,<sup>3</sup> who himself worked only with the Wassermann test, states that he is informed that the Kahn test is no longer used at the Hygienic Laboratory and at the General Hospital in Guatemala City because of frequent falsely positive reactions in cases of malaria which is very prevalent there. Sabatucci<sup>18</sup> considers that of all flocculation reactions for syphilis the Kahn test gives the greatest number of false positives in malaria. On the other hand, Smith,<sup>19</sup> working in the Amazon River Valley, reports results with the Kahn test almost identical with those which Saunders and Turner obtained with the Wassermann reaction. He did Kahn tests on the blood of 194 patients acutely sick with malaria. Of these patients, 40 per cent showed a positive Kahn. However, owing to the prevalence of clinical syphilis and yaws, the incidence of positive Kahn tests in the general population is almost exactly the same, since in 1,099 unselected cases, a positive Kahn reaction was found in 452, or 41 per cent. Of the positive Kahn reactions in malarial patients, 45 were repeated after three to six weeks, when the patients were clinically well. The Kahn tests became negative in none, and in only 2 was there a slight decrease in intensity. He concludes: "The specificity of the Kahn test is not affected by acute malaria or its febrile reaction."

Our own interest in this question was aroused by the following rather embarrassing case. Mrs. T. came to the office complaining of vague malaise, chiefly vertigo with headache, weakness, and a chilly feeling. The temperature was normal; physical examination was negative; there was no splenomegaly. The urine contained 3.5 per cent sugar, and a routine blood Kahn was strongly positive (four-plus). No blood spreads were made. A second tube of blood was sent to a clinical laboratory, well known for its careful work, which reported the Wassermann and Kline tests each four-plus. The patient and her husband were summoned to the office and the nature of syphilis explained to them and the importance of a prolonged course of treatment pointed out. Ten days later she returned stating that she had had a chill two days before and again just before coming to the office. Her tem-

day of menstruation are no more marked than those occurring during the intermenstrual period. There seems to be a slight downward trend in hemoglobin during the day. This is particularly true in Case 1, where the hemoglobin falls from 15.43 gm in the morning to 12.63 gm in the late evening during the midmenstrual period, and from 16.23 gm to 13.89 during the menstrual period. Although this small amount of data is inadequate, there is no correlation between the 3 cases which would warrant concluding that the menstrual loss affects the hourly hemoglobin deviation.

According to Dodds,<sup>17</sup> the complete menstrual cycle can be divided into four phases: (1) premenstrual period of four or five days, during which there is an increased congestion of the mucosa of the uterus, (2) menstrual period

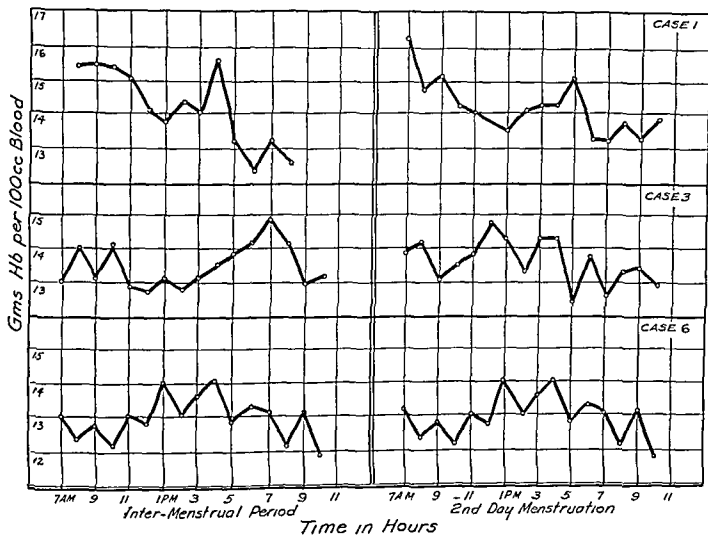


Chart 3—Curves for 3 cases showing hourly variations in hemoglobin

usually of four or five days marked by hemorrhage from the uterine mucosa and some destruction of its tissues, (3) postmenstrual period of about seven days during which the uterine mucosa returns to its original condition, and (4) intermenstrual period during which the uterus is at rest. Chart 4 shows individual graphs presenting the average hemoglobin during the four different phases of the menstrual cycle for the seven college women studied. Five days were chosen as representative of the premenstrual period and seven days of the postmenstrual period. The menstrual periods varied with the different individuals, ranging from three to five days. The total cycles varied from twenty to thirty-three days. In Cases 1, 2, 3, 5, and 7, the lowest hemoglobin value is reached during the menstrual period. In some instances, there is a

## SUMMARY

1. A review of the literature reveals a wide discrepancy regarding the occurrence of positive Wassermann and Kahn reactions in malaria. In the former test, one might possibly explain this discrepancy on the basis of variations in technic. The Kahn test is, however, so thoroughly standardized that such an explanation is not valid here. It seemed, therefore, worth while to investigate the frequency of positive Kahn tests in malarial infections.

2. In 154 cases of malaria free from the suspicion of syphilis, drawn from the practice of one of us and from the wards of the Jewish Hospital, of St. Louis, and the St. Louis City Hospital, the percentage of positive Kahn tests was 21 per cent.

3. The percentage of positive Kahn tests in the general population was 5.5 per cent in the private practice of one of us and 4.6 per cent in the medical wards of the Jewish Hospital. In the City Hospital, the percentage was 15 per cent.

4. It seems clear to us that in St. Louis, at any rate, malarial infection offers a possible source of error in the interpretation of the Kahn test.

## REFERENCES

1. Craig, C. F.: In Hazen: Syphilis, St. Louis, 1928, The C. V. Mosby Company, p. 502.
2. St. John, J. H.: The Wassermann Reaction in Malaria, *Am. J. Trop. Med.* 1: 319, 1921.
3. Curtli, W.: Syphilis in the Highlands of Guatemala, *Am. J. Syphilis* 17: 164, 1933.
4. Quoted from Hebewerth and Kop.
5. Mayr, J. K.: Die Wassermann'sche Reaktion bei Malaria, *Med. Klin.* 23: 94, 1927.
6. Schilling, C.: Protozoenkrankheiten, *Handbuch d. inn. Med.* 1: 1320, 1925.
7. Hirsch, S.: Anfall der W. R. bei Malaria, *Ztschr. f. Hyg. u. Infektionskr.* 84: 324, 1917.
8. Hebewerth, F. H., and Kop, W. A.: The Wassermann Test in Patients Affected With Malaria in the Tropics, *Am. J. Hyg.* 19: 277, 1921.
9. Fischer, O., and Günsberger, O. D.: Ueber die Ursache der positiven W. R. bei Malaria, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78: 295, 1933.
10. Nagell, H.: Unspezifische Hemmungen bei der W. R., *Dermat. Wehnschr.* 90: 795, 823, 1930.
11. Kolmer, J. A.: Truths About the Serum Diagnosis of Syphilis, *J. A. M. A.* 93: 1429, 1929.
12. Busineo, A., and Foltz, P.: Sifilide e Malaria, *Policlinico (sez. med.)* 31: 245, 1924.
13. Johnson, J. P.: Diagnosis of Syphilis in Malaria by the W. R., *J. Path. & Bact.* 24: 145, 1921.
14. Levy, M. D.: The Wassermann Reaction in Malarial Fevers, *Am. J. Trop. Med.* 1: 313, 1921.
15. McConnell, G.: Positive Wassermann Test in a Fatal Case of Estivo-Autumnal Malaria, *J. A. M. A.* 80: 1123, 1923.
16. Saunders, G. M., and Turner, T. B.: Wassermann Reaction in Malaria, *South. M. J.* 28: 542, 1935.
17. Heinemann, H.: Untersuchungen über den diagnostischen Werth der Methoden von W., S. G. und M. in Malarialändern, *München. med. Wehnschr.* 68: 1551, 1921.
18. Quoted by Curtli.
19. Smith, C. R.: Kahn Test Specificity in Malaria, *J. Lab. & Clin. Med.* 18: 396, 1933.
20. Eller, K.: Serologische Untersuchungen bei Tertiana-Impfmalaria an luesfreien Patienten, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 74: 397, 1932.
21. Wilson, R. J., and Levin, S. L.: Effect of Malaria on the Wassermann Reaction, *Am. J. M. Sc.* 191: 696, 1936.

much more pronounced drop than in others. This can be explained easily when we consider that the menstrual loss may vary from 1146 gm to 23403 gm of hemoglobin for healthy women<sup>16</sup>. Also, when it is realized that hourly fluctuations occur which are not consistently the same at corresponding hours on different days, it is not surprising that two of the cases show a slight rise in hemoglobin for the average during the few days of menstruation. It is interesting to note that the one individual studied who was definitely anemic shows the greatest drop in hemoglobin during the menstrual phase of the cycle, though she considered her menstrual periods normal. Baier and associates<sup>15</sup> found much greater menstrual losses in patients with hypochromic anemia.

In Chart 5 averages are made of the seven individual graphs presented in Chart 4. It shows that there is a peak of 14.55 gm of hemoglobin per 100 cc of blood during the intermenstrual phase of the cycle and that the lowest ebb of 13.61 gm is reached during the menstrual period, which is characterized by hemorrhage. During the postmenstrual phase there is a rise in hemoglobin up to 14.20 gm which continues to increase slightly during the next intermenstrual phase. Although the average difference between the intermenstrual and menstrual phases is only 0.94 gm in the seven cases studied, this diminution may be considered significant.

#### SUMMARY

1 The average hemoglobin for healthy women, twenty to twenty seven years of age, is approximately 14 gm per 100 cc of blood and shows daily variations ranging from 13 to 15 gm.

2 Hourly fluctuations in hemoglobin occur which are not consistently comparable at corresponding hours in different days. The hourly variations are as marked during the intermenstrual period as during the menstrual period.

3 There is usually a diminution in the hemoglobin during menstruation, the amount varying considerably with different individuals. During the postmenstrual phase of the cycle, there tends to be a rise in hemoglobin which continues to increase slightly during the intermenstrual phase.

#### REFERENCES

- 1 Williamson, C. S. Influence of Age and Sex on Hemoglobin, *Arch Int Med* 18: 503, 1916.
- 2 Lippincott, L. S. Hemoglobin and Erythrocytes in the South, *J Lab & Clin Med* 12: 679, 1927.
- 3 Murphy, W. P., Lynch, R., and Howard, I. The Value of Determinations of the Iron Content of Whole Blood, *Arch Int Med* 47: 883, 1931.
- 4 Helmer, O. W., and Emerson, C. P. Iron Content of the Whole Blood of Normal Individuals, *J Biol Chem* 104: 157, 1934.
- 5 Sachs, A., Levine, V. E., and Fabian, A. A. Copper and Iron in Human Blood, *Arch Int Med* 55: 227, 1935.
- 6 Dreyer, G. D., Bizet, H. C., and Pierce, H. F. Diurnal Variations in the Hemoglobin Content of the Blood, *Lancet* 98: 588, 1920.
- 7 Leverton, R. M., and Roberts, L. J. Hemoglobin and Red Cell Content of the Blood of Normal Women During Successive Menstrual Cycles, *J A M A* 106: 1439, 1936.
- 8 Rabinovitch, I. M. Variations of the Percentage of Hemoglobin in Man During the Day, *J Lab & Clin Med* 9: 120, 1923.
- 9 Mills, E. S. Hourly Hemoglobin Variations in Anemia, *Arch Int Med* 35: 760, 1925.
- 10 Ingersoll, W. Hemoglobin Values in Normal Adults over Periods of Time, *J Lab & Clin Med* 21: 787, 1936.

when the intranasal medication was stopped. What might result if intranasal instillations of oil are persistently and frequently used over a long period of time is illustrated in a case reported by Fischer-Wasels.<sup>10</sup> His patient died of heart failure secondary to extensive chronic pulmonary fibrosis. She was known to have taken oil intranasally for twenty years. One hundred cubic centimeters of mineral oil were recovered from her lungs postmortem.

#### REPORT OF OUR CASES\*

Two additional autopsied cases of oil aspiration pneumonia encountered in this institution during 1935 are herewith reported, not so much because of the supposed rarity of the condition, but rather in view of the interesting factors predisposing toward the aspiration of oil in each case.

**CASE 1.—History.**—W. C., an unmarried white man, fifty-eight years of age, a glass blower by occupation, was admitted to the General Hospital June 19, 1935, because of inan-

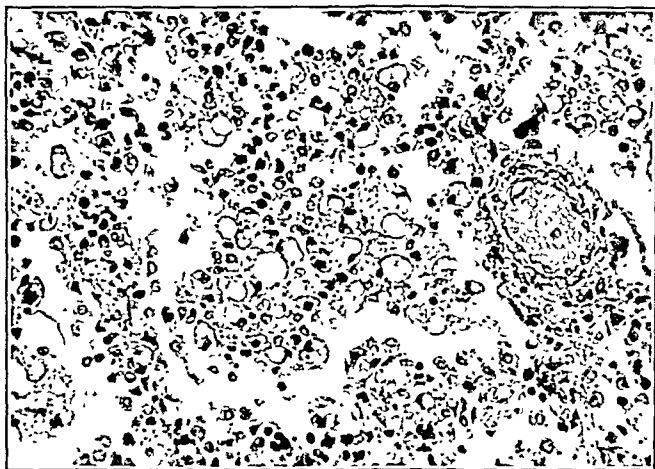


Fig. 1.—Section showing oil-containing macrophages within air sacs (Case 1). Hematoxylin and eosin stain: X250.

tion and general weakness. He gave a history of occasional "blind staggers" and "dizzy spells" during the preceding few years. Examination showed him to be emaciated, apathetic, and mentally confused. Neurologic examination was negative.

During his stay in the hospital he was up and about until two weeks before his death. He often had difficulty in swallowing either solid or liquid foods. He received haliver oil in 15 minim doses 3 times daily. On two occasions (June 15, 1935 and July 20, 1935), he was given mineral oil in 0.5 ounce doses. On August 5 he began to cough and steadily became weaker. He died Aug. 13, 1935.

**Autopsy.—Gross Findings:** The body was emaciated. In the lower lobe of the right lung were several rounded areas of consolidation, each about the size of a walnut. Each presented a grayish red, slightly convex, granular, greasy cut surface. The gallbladder contained a few stones. Both kidneys were moderately contracted, with coarsely granular cortical surfaces. (The brain was removed and examined but was entirely negative.)

**Microscopic Description of Right Lung.**—A fairly acute reaction to the aspirated oil was present in the consolidated portions of the right lung. In frozen sections, the oil was seen to be distributed in tiny droplets within swollen macrophages. The oil stained yellow

\*Irrelevant or negative clinical and laboratory findings have been omitted.

there was definite reason for suspecting the presence of syphilis Johnson<sup>13</sup> studied the serums in 74 cases of malaria by means of four different modifications of the Wassermann test. His percentage of positive reactions varied from 6 to 27 per cent. Most of the cases that gave a positive reaction were still positive when retested some time after defervescence, though the period of observation was apparently not long. At least one of these was found later to be syphilitic, and Johnson thinks that all were. In 50 cases of malaria, Levy<sup>14</sup> found the Wassermann reaction positive four times. One of the positives gave a venereal history, another with a persistent positive reaction became negative after mercury subcutaneous injection. His impression is that "when a positive Wassermann reaction is obtained during a malarial infection, we have a syphilitic infection in addition." McConnell<sup>1</sup> reports a suggestive case. A patient with a fatal pernicious malaria showed a four plus Wassermann reaction. There was no other reason for suspecting a syphilitic infection, but at autopsy a syphilitic mesoenteritis was found. Saunders and Turner<sup>16</sup> made a careful study of 1608 patients in a jaws clinic at Bath, Jamaica. Of these, 266 were listed as malarial and these gave almost the same proportion of positive Wassermanns as the general population. In most of them the reaction remained positive, whether in an attack or not. In only two was the reaction positive during the malarial paroxysm and negative at other times, both of them however, showed evidence of a previous infection with jaws. The authors suggest that in an old jaws patient, whose Wassermann has become negative, a malarial infection may again cause the latent Wassermann to become positive. Heinemann<sup>17</sup> had made a similar observation. He stated that an old syphilis or frambesia patient may have a positive Wassermann reaction made negative by malaria, to return to positive after the conclusion of treatment, while, on the other hand, a syphilitic with negative serology may have the Wassermann made permanently positive by a malarial infection.

One of our cases points a similar lesson. H. H., a paetic, had a four plus Kahn in his blood serum in 1931 with a three plus Kahn and a paetic gold curve in the spinal fluid. Fairly vigorous treatment resulted in negative serology without neurologic improvement. In July, 1934, he entered the hospital for malarial therapy. At the time, the blood Kahn was negative, the spinal fluid showed a negative Kahn but 74 cells per cmm. The day after his first malarial paroxysm, the blood Kahn became three plus, the Wassermann reaction three plus. The following day both of the tests were four plus, and have remained so ever since.

The most recent publication on the subject is that of Wilson and Levin,<sup>21</sup> who reviewed the material at a large general hospital in Charleston, S. C. Of 262 cases of malaria 192 gave a negative and 70 a positive Wassermann. Of the 70 positive reactions, 13 were definitely not syphilitic as was shown not only by the history and physical findings, but also by the fact that in all of them the serology became negative after defervescence. These 13 false positive reactions represent about 5 per cent of the total number of malaria cases, about 4 per cent of the total number gave a falsely positive Wassermann reaction and less than 3 per cent a falsely positive Kahn reaction. As will be seen, these are much lower figures than we found in our own cases. Another in

On Sept. 6, 1935 he sustained a severe cerebral accident, causing left-sided hemiplegia. He became comatose and died Sept. 7, 1935.

*Autopsy.—Gross Findings:* There was an extensive right-sided cerebral hemorrhage; severe atherosclerosis was present in the cerebral and coronary arteries and aorta. A patchy bronchopneumonia was present in the lower lobes of both lungs and in the right middle lobe. The larger branches of each pulmonary artery contained loose embolic chunks of thrombus material. The heart was dilated; mural thrombi were present in the right auricle. The kidneys were slightly contracted.

*Microscopic Description of Lungs.*—Several of the consolidated foci presented an acute seropurulent or serofibrinopurulent exudate. A block from another place presented an early reaction to oil, quite similar to that described in Case 1. In addition, there were several foci of fibrotic reaction, much older than any seen in the first case. In these areas, the lung architecture was replaced by dense fibrous tissue which was honeycombed by large and small oil droplets (Fig. 2). Each oil droplet was bordered by foreign body giant cells. In frozen sections, all of the oil stained yellow with scarlet red and failed to react with osmic acid and Nile blue sulphate.

In this case, the appearance of definite columnar epithelium lining the alveoli, which were filled with lipophages, was more pronounced than in the preceding one.

*Diagnosis:* Cerebral hemorrhage; acute and chronic bronchopneumonia (mineral oil aspiration); pulmonary embolism; generalized arteriosclerosis.

#### COMMENT

The intrapulmonary lesions observed in both of these patients were quite similar morphologically to those previously described by other writers, notably Laughlen,<sup>1</sup> Pinkerton,<sup>2,3</sup> and Graef.<sup>7</sup> All of the stages of pulmonary reactivity to oil were encountered in one or the other of these two cases, ranging from intraalveolar phagocytosis of fine oil droplets early in the process, to the formation of densely fibrous foreign body granulomas about large oil droplets late in the disease. In both cases the oil found in the lungs gave staining reactions typical of mineral oil. This would indicate that in Case 1 the haliver oil did not gain entry into the patient's trachea, even though he had had many doses of it, and only two of mineral oil. An explanation may lie in the fact that haliver oil is more irritant to the pharynx and trachea than mineral oil. In each case there was an associated septic bronchopneumonia, an expected finding in the light of previous reports.

From a practical standpoint, a valuable lesson is contained in these two cases. Neither patient suffered from demonstrable paralyses during the time the oil was administered. The only clinical contraindication to the oral administration of mineral oil presented by these two patients was their difficulty in swallowing. This impairment of the swallowing act was not marked, as neither had to be hand fed. One patient's (E. L.) practice of holding the oil in his mouth for a time in order to avoid swallowing it, certainly may have further disposed toward the aspiration of oil in his case. It is probable that the incidence of oil aspiration pneumonia could be materially lessened if physicians would routinely question patients regarding dysphagia before prescribing mineral oil for laxative purposes.

The attention of practicing physicians is not as yet sufficiently focused upon the problem of oil aspiration pneumonia, not only as a morbid entity, but, just as important, as an insidious and dangerous clinical ailment, which like many other diseases, if unsuspected remains undiagnosed. Oil aspiration

perature was 105° F, there was no splenomegaly, but the blood contained the parasites of tertian malaria. Quinine therapy was instituted. Two weeks after defervescence, the Kahn test was still slightly positive (one plus), but two weeks later it had become negative. Since then, at two and six month intervals, the Kahn test has remained negative, and the patient has been well except for a mild diabetes.

Malaria is not very prevalent in St. Louis, but during the past four years, 61 nonsyphilitic cases of malaria came under observation in the private and hospital practice of one of us. The other during 1935 studied 93 cases in the wards of the City Hospital in which Kahn tests could be done. The great majority were of the tertian type, a few being estivo autumnal. All cases in which there existed even a suspicion of syphilis were carefully excluded. Of these 154 cases, 37 gave a positive Kahn reaction (usually a four plus) making over 21 per cent positives. In 28 of the patients with a positive Kahn a Wassermann test was also done. 22 of the set gave a positive reaction, 6, a negative. No case was seen in which the Wassermann test was positive while the Kahn test was negative.

Of the 37 malarial patients with a positive Kahn test, 13 could be followed up serologically. In all of them, the Kahn test became negative after quinine therapy had caused cessation of fever. In 4 cases, the Kahn test had become negative within eight days, in all of them within fifteen days.

One case is interesting from another angle. F. C. came to the office with a history very suggestive of malaria. She had taken some quinine but had discontinued it on account of vomiting. She was running a little daily fever and had a large spleen. The blood showed a marked secondary anemia, but repeated thick and thin spreads failed to reveal the presence of malarial parasites. The blood Kahn test, however, was three plus. Under quinine medication, the fever ceased, the spleen became smaller and finally could no longer be felt, the Kahn test two days after defervescence was one plus, fifteen days later negative, and has remained so. It would seem that occasionally a positive Kahn test might aid in the diagnosis of malaria.

The most striking testimony against the view that malarial infection will cause a positive Kahn or Wassermann in a considerable proportion of cases has been that of Smith and of Saunders and Turner, who found the percentage of positive serology in malaria identical with that in the general population. In our group of patients, this was not the case. In the private practice of one of us, it has been routine to do a Kahn test on the blood serum of each new patient. During the past two years, out of 679 such patients, 37 showed a positive Kahn test, making 5.4 per cent. In the medical wards of the Jewish Hospital, of St. Louis, from which some of our malarial cases were drawn and where the same rule is observed, there were 411 admissions during the past year with 19 positive Kahn tests, a proportion of 4.6 per cent. In the City Hospital, of St. Louis, the proportion of positive Kahns is higher, being about 15 per cent of the total population. However, among the cases of malaria there, without regard to the presence of syphilis, the percentage of positive Kahn tests was 31 per cent. The difference, or 16 per cent, may reasonably be regarded as indicating the incidence of positive Kahn tests due to malaria.

## STUDIES ON CONSTITUTION AND PEPTIC ULCER\*

### IV. SALIVARY SECRETION TEST IN PEPTIC ULCER PATIENTS AND NORMAL SUBJECTS

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EPPINGER and Hess of Vienna applied the conception of "vagotonia" to peptic ulcer patients on the basis that the majority of them presented symptoms attributable to a hyperactivity of the parasympathetic nervous system, but admitted that in a number of ulcer patients mixed symptoms were to be seen. They injected peptic ulcer patients with pilocarpine, and reported an increased salivary secretion in response to this drug.<sup>1</sup> Von Bergmann and his school elaborated upon the idea of Eppinger and Hess, and supplanted the conception of vagotonia by that of "autonomic imbalance," by which they characterized ulcer patients.<sup>2, 3</sup> Increased salivation in patients with peptic ulcer has been described by several authors.<sup>3-5</sup> None of these investigators, however, have made a comparative study on the salivary secretion of ulcer patients with that of a similar group of normal persons.

#### MATERIAL AND METHODS

All subjects were white adult males between the ages of twenty and sixty-six years. Thirty normal individuals free of any gastrointestinal complaints were selected, along with 34 peptic ulcer patients picked at random from our gastrointestinal clinic. Two of the patients suffered from gastric ulcer, the remainder from duodenal ulcer. The ulcers were in various stages of activity. The diagnosis had been established clinically, roentgenologically, and by other laboratory tests, in several by operation; no case was included in which the diagnosis was doubtful.

The individuals presented themselves for the test in the morning, two or three hours after an ordinary breakfast. A subcutaneous injection of 1 mg. of pilocarpine nitrate for every 30 pounds of body weight was given. The expectorated saliva was collected for an hour following the injection, and the volume measured. In all cases the peak of the secretion curve occurred in the second quarter-hour period. No untoward reactions from the pilocarpine injection were observed, but nearly all subjects experienced some flushing and perspiration.

The data were tabulated in the form of a correlation table, the coefficient of correlation between the volume of secretion and age being determined according to the formulas of Pearl.<sup>7</sup> Statistical means and the significance of their differences were computed by the method of Fisher.<sup>8</sup>

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## OIL ASPIRATION PNEUMONIA\*†

### REPORT OF TWO AUTOPSYD CASES IN ADULTS

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**INTRODUCTION**—Attention has been directed to oil aspiration pneumonia on this continent largely through the contributions of Laughlen,<sup>1</sup> Pinkerton,<sup>2</sup> Rabimovitch and Lederer,<sup>4</sup> Ikeda,<sup>5</sup> and Graef.<sup>7</sup> These workers have reported a total of 31 autopsied cases among which 26 occurred in infants or children and 5 in adults. Perusal of the literature will reveal a number of additional reports, most of them concerning clinical cases.

**Etiologic Factors**—The various circumstances or mechanisms predisposing to the development of this disease have been discussed thoroughly by the authors mentioned above. A point in common among many of the reported cases is the presence of some interference with the act of swallowing, due either to local or central causes. Infantile marasmus, stuporous states, and central nervous system lesions are often contributory. Any depression of bulbar function sets the stage for aspiration of oil into the lungs. The methods by which the oil is introduced differ, but it is known that intranasally instilled oily medicaments and orally administered cod liver oil or mineral oil account for a high percentage of the cases reported. The forced intake of cod liver oil into the throats of resistive children has been responsible for the development of oil aspiration pneumonia. Fatty substances present in foods have not infrequently been etiologic in infants.<sup>8</sup> Although certain writers have stressed the point that oil aspiration pneumonia is not a disease liable to affect healthy people, it should not be thought that this is a hard and fast rule. The existence of impaired deglutitive function is not absolutely necessary in order that oil be aspirated, particularly when the oil is taken intranasally. Graef<sup>7</sup> has called attention to the fact that mineral oil is non-irritating and does not excite the cough reflex. The addition to it of mildly anesthetic substances such as menthol further lessen its irritative potentialities. Along this line, the experience of Tchertkoff and Ornstein,<sup>9</sup> as set forth in a recent clinical report, is interesting. These authors reported 10 cases of "bronchopulmonary disease" which they attributed to the intranasal use of oily substances. Eight of their cases were encountered during the space of a few months of consultant office practice. The authors warned against the indiscriminate use of intranasal oil and stressed the importance of questioning chronic lung sufferers as to whether or not they used nasal medication, in order to detect the disease more frequently. All of their patients improved.

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†This term suggested by Ikeda is used here to include any lung disease attributable to the aspiration of oils or fats.

The work presented tends to show that peptic ulcer is not a local disease, but probably a systemic one. In another paper of this series, it was shown that the supposedly healthy members of ulcer families have an abnormal gastric secretion,<sup>10</sup> so that constitutional differences do exist between ulcer patients and their relatives and normal individuals.

Our results are not in favor of the theory of vagotonia, but support the concept of autonomic imbalance, somewhat because a disturbance (or imbalance) in the parasympathetic system itself may explain our findings.

#### SUMMARY

The salivary response to pilocarpine was determined in a group of 30 normal persons and 34 ulcer patients. In neither group was any relation of volume of secretion and age seen. The salivary secretion of the ulcer group was significantly lower than that of the normal control group.

We are indebted to Dr. T. O. Yntema, School of Business, University of Chicago, advice in the statistical treatment of the results.

#### REFERENCES

1. Eppinger, H., and Hess, L.: Vagotonia (Trans.). Nervous and Mental Disease, 37: 541, 1926.
2. Von Bergmann, G.: Das spasmogene Ulcus. Ztschr. f. klin. Med. 1913.
3. Westphal, K., and Katsch, G.: Das Nervensystem. Med. u. Chir. 60: 391, 1913.
4. Winkelstein, A.: Gastric and Duodenal Ulcer. J. Clin. Med. 37: 541, 1926.
5. Petré, K., and Thorling, I.: Untersuchungen über den Sympathikotonus. Ztschr. f. klin. Med. 1913.
6. Platz, O., in Müller. Die Lebensnerven, Nervensystems. Ztschr. f. klin. Med. 1913.
7. Pearl, R.: Medical Biometry and Statistics, 3: 90, 1936.
8. Fisher, R. A.: Statistical Methods for Research Workers, 3: 90, 1936.
9. Neeheles, H., and Maskin, M. H.: Studies in Gastric Secretion in Normal Persons and in Peptic Ulcer. J. Clin. Med. 3: 90, 1936.
10. Meyer, Jacob, Maskin, M. H., and Neeheles, H.: Gastric Secretion in the Healthy and in the Ulcer Patient. J. Dig. Dis. & Nutr. 3: 501, 1936.

with scarlet red and failed to react with either mile blue sulphate or osmic acid. In paraffin sections, the oil containing macrophages or "lipophages" were easily identified by their vacuolization (Fig 1). Many of the cells were greatly swollen. In some almost no cytoplasm remained. Nuclei were often eccentric. These oil containing cells were grouped within the air sacs, often in company with one or more other exudative elements chiefly fibrin, serum, and polymorphonuclear leucocytes. Many air sacs were completely filled solely with lipophages. In others there was an admixture with fibrin or polymorphonuclear leucocytes or both. In yet other areas the response was more or less cellular. In the peripheral portions of the consolidated areas, the exudate was predominantly fibrinopurulent. An interesting finding, several times noted previously in this disease was that in many of the alveoli which contained lipophages, the alveolar epithelium was easily demonstrable being made up of deep staining cells of cuboidal to low columnar type.

Although for the most part the reaction to the oil was acute in several areas concentric formations of young fibrous tissue were seen enclosing large droplets of oil which were intimately clothed by foreign body giant cells. Other large droplets of oil which seemed to have resulted from the agglomeration of lungs' lipophages were surrounded by deep staining

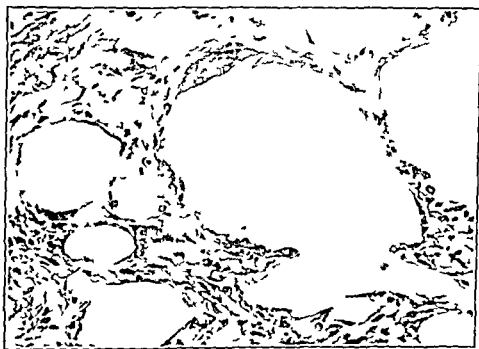


Fig. 1.—Section showing foreign body granulomatous reaction to large oil droplets in scarlet lung (Case 1). Hematoxylin and eosin stain.  $\times 200$

fresh macrophages which seemed to be in a process of fusion to form foreign body giant cells. These droplets had not yet excited fibrous response.

**Diagnosis.** Acute bronchopneumonia (mineral oil aspiration), invasion, chronic glomerulo and interstitial nephritis.

**CASE 2—History.** E. L., a married white man, sixty one years of age, a bug exterminator by occupation, was admitted to the General Hospital Oct. 15, 1934. In addition to chronic arthritis of both knees, he complained of shortness of breath. He remained in the hospital for nearly a year, during which time he was usually able to be up and about. He showed mental confusion, continually drooled at the mouth, and had difficulty in swallowing. He was frequently constipated, but when laxative tablets were given him, he would hold them in his mouth until he felt he was not watched, when he would spit them out. On June 11, 1935 he suffered a mild cerebral accident characterized by muscular twitchings of the face and both upper and lower extremities. There were no residual signs. From this time on, he was given mineral oil in one ounce doses when indicated, rather than pills or tablets. Up to the time of his death he had received at least 10 such doses, this medication too he held in his mouth and later spit out if not carefully watched.

est positions of the cervical glands. Only rarely were the deeper uterine tissues found to be involved, since its periodic change and power of regeneration, as the result of ovarian hormone stimulation, protect this organ, in some degree, from an extensive infection. The tubes and ovaries act as reservoirs or foci par excellence for the gonococcus. Latency of the infection is therefore a frequent sequel in both the male and female genital organs.

As smears and cultural procedures are not always of value in the diagnosis of latent gonorrhea, the complement fixation test that was discovered in 1906 by Müller and Oppenheim,<sup>4</sup> and, independently of them, by Bruck,<sup>5</sup> becomes useful as an aid in diagnosis of gonococcal infection. Many investigators in this country, among them Teague and Torrey,<sup>6</sup> Wollstein,<sup>7</sup> Schwartz and McNeil,<sup>8</sup> and a great number in Europe, including Vannod,<sup>9</sup> Krumbein and Schatilloff,<sup>10</sup> and many others were among the pioneers of this new laboratory method. Because of the technical difficulties and errors in interpretation, the use of the complement fixation test as a routine laboratory procedure was delayed for more than twenty years. The main difficulty in the past has been the production of a suitable antigen that had a wide range of reactivity. Attempts to improve the gonococcus complement fixation technic have been only moderately successful, since it is far more delicate than the Wassermann test. The reaction has been improved in many ways but much more still remains to be done. It was not until seven years ago that the European countries accepted it as a routine method of diagnosis. This aroused in this country a new interest in the reaction. Considering the American literature of the last five years, the reader is confronted with different opinions in regard to the clinical value of the reaction. There are some workers who think that this test has a very limited, if any, clinical value (Herrold,<sup>11</sup> Jacoby,<sup>12</sup> Bucher,<sup>13</sup>) while others (Kolmer,<sup>14</sup> Swan,<sup>15</sup> Barringer and her associates,<sup>16</sup> Brunet and Levine<sup>17, 18</sup>) have reached the opposite conclusion. Barringer, Strauss and Crowley mention the importance of this method for the determination of a cure. This matter has been under discussion for many years among the European investigators. In a paper on this subject, published by me together with Gräfenberg<sup>19</sup> in 1925, it was pointed out that this test was probably helpful in making more certain the establishment of cure. In spite of extensive work this problem still remains unsettled.

The purposes of the study reported here are: (1) to help the clinician in the interpretation of the gonococcus complement fixation test with respect to its clinical value and to demonstrate the limitations of the reaction; (2) to prove that this test is helpful in the determination of cure.

#### EXPERIMENTAL

The technic which has been used in these studies is the same as that which has already been published. The main principles of this procedure will be repeated here.

*Antigen.*—The gonococci (about 12 strains) were originally cultivated on Levinthal's cooked blood agar, but are now grown on the modified filtrated cooked blood agar, suggested by Bayne-Jones. The age of the strains is of minor importance, since old laboratory strains show the same antigenic power as young

pneumonia is not necessarily fatal. All of the patients reported by Tchertkoff and Ornstein<sup>9</sup> improved when intranasal oil medication was stopped. In all probability, individuals may recover from mild or subclinical lung damage resultant from administration of oil by mouth, without the condition's ever having been recognized. The suggestion of Rabinovitch and Lederer<sup>4</sup> that sputum from suspected cases be examined for oil containing monocytes seems a good one. This procedure along with careful history taking and adequate x-ray studies should lead to more frequent clinical recognition of this interesting and often unnecessary disease.

#### CONCLUSIONS

1 Two cases of oil aspiration pneumonia in adults are herewith reported with autopsy findings. The pulmonary lesions observed in each instance correspond well with those described by the writers mentioned previously.

2 Oil aspiration pneumonia is often preventable. Care should be taken that mineral oils as a laxative should be prescribed only for those patients in whom there is no interference with the act of swallowing. The frequent use of nose oils to judge from certain recent contributions to the literature, is also fraught with danger, even where there is no impairment of the deglutitive function.

3 If increased attention is directed toward oil aspiration pneumonia, it will be recognized more frequently, both clinically and at autopsy.

#### REFERENCES

- 1 Laughlen, G. F. Pneumonia Following Naso-Pharyngeal Injections of Oil, *Am J Path* 1: 407, 1925.
- 2 Pinkerton, H. Oils and Fats: Their Entrance into and Fate in Lungs of Infants and Children, *Clinical and Pathologic Report*, *Am J Dis Child* 33: 259, 1927.
- 3 Pinkerton, H. Reaction to Oils and Fats in Lung, *Arch Path* 5: 330, 1928.
- 4 Rabinovitch, J., and Lederer, M. Lipoid Pneumonia, *Arch Path* 17: 160, 1934.
- 5 Ikeda, K. Pathology of Oil Aspiration Pneumonia (Lipoid Pneumonia), *Am J Clin Path* 5: 89, 1935.
- 6 Ikeda, K. Oil Aspiration Pneumonia (Lipoid Pneumonia), *Clinical, Pathologic and Experimental Consideration*, *Am J Dis Child* 49: 985, 1935.
- 7 Graef, I. Pulmonary Changes Due to the Aspiration of Lipids and Mineral Oil, *Scientific Proceedings of the Thirty-Fifth Annual Meeting of the American Association of Pathologists and Bacteriologists*, *Am J Path* 11: 862, 1935.
- 8 Pierson, J. W. Pneumonia Due to Aspiration of Lipoids, *J A M A* 99: 1163, 1932.
- 9 Tchertkoff, J. G., and Ornstein, G. G. Bronchopulmonary Disease Attributed to the Use of Intranasal Instillations of Oily Substances, *Quart Bull Ser View Hosp* 1: 139, 1936.
- 10 Fischer-Wasels, B. Todliche Lungenschrumpfung durch Gebrauch von Mentholol, *Frankfurt Ztschr f Path* 44: 412, 1933.

half an hour, and then stored in the ice box for twenty-four hours. Since freshly inactivated serum shows an increased lability of its reactive substances and therefore might yield false serologic results, the test should not be made on the day of inactivation. A serum used more than three days after being taken from the patient may give nonspecific reactions. The same is true for serums from patients who have recently been immunized with gonococcus vaccine; a period of at least six weeks must elapse before results, not influenced by the immunization, can be expected.

*Amboceptor.*—The titration of the amboceptor is carried out in dilutions of 1:200, 1:400, et., to 1:25,600. Complement taken twenty-four hours previously from a healthy male guinea pig in a dilution of 1:10 and sheep's blood in a dilution of 1:20 are added. The different reagents are always used in the same amounts (0.25 c.c.). Each tube contains a total of 1.25 c.c.; the difference, due to lack of serum and antigen in this preliminary test, is made up with normal saline. Two control tubes containing no amboceptor are set up in order to exclude the possible hemolytic action of the complement or the saline. This preliminary test is incubated at 37.5° C. for one hour. The first reading is made after twenty minutes, the final one after one hour. The unit of amboceptor is taken either as that dilution in which complete hemolysis occurs in twenty minutes, or one-fourth of that dilution in which complete hemolysis occurs in one hour; the lowest dilution showing hemolysis is taken as the titer. For example: if after twenty minutes complete hemolysis occurs with 1:800 dilution of amboceptor, after one hour with a 1:3,200 dilution, the unit is 1:800. Or, if hemolysis occurs after twenty minutes in a dilution of 1:400, after one hour with a dilution of 1:3,200, the unit is 1:400. Or, if hemolysis takes place after twenty minutes at a dilution of 1:1,600 and after one hour at a dilution of 1:3,200, the unit is 1:800. The purpose of the double reading at different intervals of time is to exclude the hemolyzing power of the complement itself.

*Main Test.*—In the main test three tubes are used for each serum. The first, which acts as a serum control contains no antigen but double the amount of serum (0.5 c.c.). The second and third tubes contain all the constituents of the first tube with the addition of the antigens which differ in each tube. Known positive and negative serums are always included in the main test as controls. After adding complement, the test is incubated at 37.5° C. for one hour. The hemolytic system (0.5 c.c.) is then added to each tube. Readings are made as soon as the negative serum controls, with and without antigen, are hemolyzed. This may take place in from twenty to sixty minutes. With readings at the moment when the controls are hemolyzed, the weak positive reactions are not overlooked.

The complement fixing power between antigen and antibody is the weak point in this test a fact which can readily be shown by comparison of the gonococcus complement fixation reaction with the more stable Wassermann test. The Hamann combined system, reported by Thomson, Hamann and Park,<sup>22</sup> attempts to make the test more stable by titrating the fixing power of the complement.

The tests reported in this paper were carried out, using the technic which has been described above and the results recorded as + + + + (strongly positive);

## RESULTS

From Table I it appeared that the older group of ulcer patients (forty one to sixty six years) secreted more saliva than the younger group (twenty one to forty years), and that no such difference existed between the normal groups. By using Fisher's formula<sup>8</sup> for small series it was found that the increase of salivary secretion with age in the ulcer group was not significant. Therefore, the combined age groups of the ulcer series were compared with the combined

TABLE I

AGE IN YEARS	NO OF SUBJECTS		SALIVARY SECRETION IN CC	
	ULCEP	NORMAL	ULCEP	NORMAL
21-40	13	1	8	115
41-66	21	1	10	118
21-66	34	2	$9.1 \pm 4$	$116 \pm 7.3$
Difference of the means of both groups and standard error of the difference			$21.9 \pm 9.7$	

age groups of the normal series. The means of all ulcer patients and of all normal persons determined statistically were 9.1 and 116 cc, respectively. The difference of the means of the normal and ulcer group was  $21.9 \pm 9.7$  cc. Since this difference was more than twice its standard error, it had to be considered significant, and it must be concluded that peptic ulcer patients secrete less saliva following pilocarpine than normal subjects.

## DISCUSSION

The finding that the patient with peptic ulcer secretes less saliva to pilocarpine stimulation certainly speaks against the theory of generalized vagotonia. Our results do not necessarily disprove that ulcer patients may have increased salivation (Winkelstein<sup>4</sup>) but the probability is, that it is not so. In a previous paper of this series, we reported that gastric appetite secretion was higher in acidity in ulcer patients than in normal subjects.<sup>9</sup> It was pointed out that the greater acid response to appetite stimulation in ulcer patients could hardly be used diagnostically, because the individual results were too scattered and only statistical treatment proved that there was a significant difference. The same is true in the present paper. The significance of the difference in salivary secretion between the normal and ulcer group had to be proved statistically, and therefore salivary response to pilocarpine cannot be recommended as a simple diagnostic test.

It remains an open question why the ulcer patient secretes less saliva than the individual without an ulcer. Many patients with gastric ulcer display hypermotility of the stomach and many patients with peptic ulcer of the duodenum show gastric hypermotility and hyperacidity. It is probable that this hyperactivity is due to impulses reaching the stomach through the vagi or by a humoral mechanism. One may assume, then, that certain centers in the midbrain are in a state of hyperactivity (centers for gastric secretion and motility), and that other centers are not affected, or even may be depressed (salivation center). Since salivary secretion is a complex process, one must think also of psychic inhibition or of peripheral reflex inhibition.

earlier than this by improving the technic of the test. Price<sup>23</sup> tried to demonstrate this fact by immunizing himself, patients and animals with a gonococcus vaccine. He found a strongly positive serologic reaction present by the tenth day. However, the mechanism of antibody production seems to be different from that of natural infection. The problem involves the question as to whether or not it is possible to use this test in making a diagnosis of early gonorrhea.

TABLE I  
RESULTS OF COMPLEMENT FIXATION TESTS IN VARIOUS CASES

REACTION	++++		+++		++		+		0	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
<i>A. Males:</i>										
Anterior urethritis										
50 cases	2	4	2	4	7	14	1	2	38	76
Posterior urethritis										
83 cases	10	12.05	16	19.27	22	26.50	20	24.10	15	18.07
Prostatitis										
7 cases	2	28.57	2	28.57	2*	28.57	-	-	1	14.29
Epididymitis										
7 cases	4	57.14	1	14.29	2*	28.57	-	-	-	-
<i>B. Females:</i>										
Cervicitis										
30 cases	7	23.33	5	16.66	3	10.00	2	6.66	13	43.33
Adnexitis										
18 cases	11	61.11	2	11.11	3	16.66	1	5.55	1†	5.55
Bartholinitis										
4 cases	1	25.00	-	-	2	50.00	1	25.00	-	-
<i>C. Arthritis:</i>										
7 cases	4	57.14	1	14.28	-	-	-	-	2‡	28.57
<i>D. Chronic:</i>										
9 cases	2	22.22	2	22.22	3	33.33	2	22.22	-	-

\*Changed later to +++++.

†Changed after twelve days to +++++.

‡One: ten days later to +++++; second, after eighteen days to ++.

Cohn and Gräfenberg pointed out this possibility in 1925, but after further studies the idea was dropped, because of the fact that an early formation of antibodies in gonococcal infections could be demonstrated only irregularly and exceptionally. In the present study serum from a patient suffering from an acute gonorrheal discharge for five days was found to give a positive reaction; the clinical diagnosis was acute infection or recurrent. Nevertheless, because of a strong suspicion that these symptoms represent an example of the early serologic method has no practical importance, determined by bacteriologic means.

Another frequent reason for the atypical reaction is the nonabsorption of antigen due to the surface of the urethral or cervical mucous membrane, surprising that the largest number of atypical reactions were obtained with anterior urethral and with cervical because the gonococcus spreads on the mucous membrane, which have a great amount of antigen will be produced. On the contrary, with

# LABORATORY METHODS

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## THE GONOCOCCUS COMPLEMENT FIXATION TEST

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**S**EVEN years before the discovery of the gonococcus by Neisser, Noeggerath<sup>1</sup> attracted the attention of physicians to the importance of latent gonorrhea in the female in his masterly clinical study entitled *Die latente Gonorrhoe des weiblichen Geschlechts*. Since the etiologic agent of gonorrhea was as yet unknown, Noeggerath's statements were based only on clinical observations. The difficulty in diagnosis encountered by this worker is obvious.

With the discovery of the gonococcus, the difficulty in diagnosis of subacute and chronic cases of gonorrhea was further emphasized. Frequent microscopic examinations sometimes had to be made before gonococci could be found in smears. Zill, as cited by Brandstруп,<sup>2</sup> states that gonococci may be demonstrated in females in 40 per cent of chronic cases after an examination of seven smears, in some instances it has been possible to demonstrate the gram negative diplococci only after examining 70 to 80 smears.

With the adoption of the culture method as routine, there seemed to be some increase in the efficiency of diagnosis of gonorrhea. It is evident that material containing only a few organisms will yield a greater number of positive results when examined by the cultural than by the microscopic method, because of the increase in numbers due to growth in a suitable medium. An experienced worker in this field knows that there are instances in which only the smear may be positive while the repeated cultures fail to show growth of the gonococcus. Therefore it is essential to employ both methods as aids in the diagnosis of gonorrhea.

Even these laboratory procedures are limited in their usefulness, particularly in the cases in which the gonococci have penetrated into the tissues and surrounding structures so as to disappear completely from the discharge.

The lacunae of Morgagni and the glands of Littlé of the male anterior urethra, Cowper's glands, the prostate, the seminal vesicles, or the epididymis frequently become encapsulated foci of gonococci. Depending on the stage of inflammation, these foci may be more or less surrounded by an inflammatory cell and tissue reaction or may even form an abscess. This type of latent gonococcal infection is also known as a frequent complication of the disease of the female sex organs. R. Schroeder,<sup>3</sup> among others, has demonstrated gonococci in the deeper layers of the altered mucous membranes of the cervix, concealed in the interstitial infiltrations of round and plasma cells lying between the deep

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suspicious cases showed a +++; 1 a ++ reaction; in 1 of them the gonococcus was found on culture in the Bartholinian gland. The 2 other cases could not be established bacteriologically, but a purulent discharge, temperature in the puerperium, and swelling of the joints in 1 patient, and a discharge, temperature and condylomata acuminata in the other made the clinical picture suspicious.

Four cases of sterility in female patients with negative history and bacteriologic findings gave negative reactions.

The serums of 25 children, of whom 10 showed no evidence of a gonococcal infection, were tested. Nine gave a negative serum reaction and 1 a + reaction; this child was suffering from a septicemia of unknown origin. Of the 15 children who had positive smears and cultures of the vagina or rectum or both, 3 gave strongly positive, 6 a weakly positive, and 6 a negative reaction. These results are similar to those described previously by Cohn and Rosowsky.<sup>24</sup> In these cases no explanation could be found for the discrepancies in the serologic results in children with an identical clinical picture and apparently the same constitutional make-up. The age may be an important factor in this group, since the strongly positive reactions were found in children of three or more years old while the younger ones showed a weak or negative reaction.

*The Determination of Cure.*—The problem as to whether the gonococcus complement fixation test is useful in the determination of cure has been intensively discussed in European countries for a long time. The surprising fact that positive serologic results were obtained in patients who had been declared cured for many years, and that negative serum reactions were followed by positive bacteriologic findings tended to discredit the test.

Due to the fact that the above-mentioned positive reactions were found in clinically cured cases in which the gonococcal infection had occurred ten to twenty years previously, a persistence of complement fixing antibodies without continued stimulation by an antigen was assumed. The adoption of this idea seems surprising, because the same assumption is never made in the Wassermann reaction, in which similar observations can be noted. Cohn<sup>25</sup> attempted to clarify this point by immunizing normal persons with a gonococcus vaccine. He produced a positive reaction in such persons and found that the antibodies disappeared in from twenty-six days to three months after the vaccination; even if the mechanism of the production of complement fixing antibodies by immunization might be different from that of natural infection, these experiments show, obviously, the disappearance of antibodies after the elimination of the antigenic effect. It is also significant that the persisting positive reactions were found mainly in cases with gonococcal complications which frequently showed a recurrence of the disease. Further, there were cases in which the positive reaction became negative after the clinical cure. All of these facts, taken together, indicate that a continuing positive reaction is more than just the remnant of a serologic reaction.

Unfortunately, the number of serums from patients who were considered as clinically cured, was small. Nevertheless there were cases which show clearly the disappearance of complement fixing antibodies after cure.

ones. It is very important to have strains with a wide antigenic reactivity. From the experiments of Teague and Torrey and the more recent study of Tulloch,<sup>20</sup> it is known that a main or 'predominant' type of the gonococcus causes infection in 72 per cent of all cases. The remainder of the organisms are divided into four smaller groups, which are less frequently the causes of infections (28 per cent). It has been reported in a previous paper<sup>21</sup> that a predominant strain, which Tulloch received from Teague and which was sent to me, had nearly the same antigenic reactivity in Berlin as in this country and in England. So, it probably is sufficient for practical purposes to use an antigen made with several strains in order to insure the presence of the predominant type in the antigen and to avoid typing of the gonococci.

Forty eight or seventy two hour cultures of gonococci, which have previously been identified, are examined for purity. Five cubic centimeters of normal saline are added to each Petri dish culture and the colonies carefully suspended by streaking the loop over the surface of the medium. To avoid nonspecific action of the antigen, care is taken not to include any of the agar medium. The suspensions, to which 0.5 per cent phenol is added, are collected in liter bottles and closed by glass or rubber stoppers, since cork or cotton plugs may influence the specificity of the reaction. It is advisable to store the bottles in the ice box for about three months and to shake the suspensions occasionally in order to "ripen" the antigen properly.

After three months the antigen is ready for titration. To secure a constant titer the suspension must be shaken each time before use. The gonococcus antigen is mixed in amounts ranging from 0.1 to 0.025 c.c. with known positive and negative human serums, diluted 1:5, as well as with normal saline as a control. After adding complement in a dilution of 1:10 to each tube, the whole test is incubated at 37.5° C. in the incubator for one hour. The use of the incubator is preferred because the reaction in the water bath proceeds too rapidly. After addition of the hemolytic system, the test is reincubated and read as soon as the control tubes (serum without antigen, and negative serum with antigen) are hemolyzed. The titer of the antigen is considered as the lowest dilution which gives a reaction with the positive serum and complete hemolysis with the negative serum. In most cases the antigen-saline test tube will also be hemolyzed in the corresponding dilution, because of the fact that the anticomplementary quality of the antigen is neutralized by the presence of serum, this control is not regarded as essential. The antigen must be tested with many serums before the exact titer is determined. As a rule the titer lies between 0.06 and 0.03 c.c. for a total volume of 0.25 c.c. It must be emphasized that only human serums should be used for titration, since immune serums of animals contain antibodies in higher titer and therefore require less antigen for the test. The titer determined by the above method usually remains constant. An increase of the antigenic power within the first six months, followed by a slight decrease, may occur.

*Serum to Be Tested*—The patient's serum is obtained in the same manner as for the Wassermann test. If the blood is to be shipped, it is also essential to close the glass tube with a rubber stopper. Soon after the arrival at the laboratory the blood must be centrifuged, the serum inactivated at 56° C. for

## REFERENCES

- 1 Noeggerath Die latente Gonorrhoe des weiblichen Geschlechts, Bonn, 1872
- 2 Brandstrup, E. Gonorrhoea Complement Fixation Puerperium, Acta obst et gynec Scandinav 12 125, 1932
- 3 Schroeder, R. Grundsatzliches zur Behandlung der Zervigonorrhoe, Dermat Wehnschr 92 757, 1931
- 4 Muller, R., and Oppenheim, M. Ueber den Nachweis von Antikörpern im Serum eines an Arthritis gonorrhoea Erkrankten mittels Komplementablenkung, Wien. klin Wehnschr 19 891, 1906
- 5 Bruck, C. Über spezifische Immunkörper gegen Gonokokken, Deutsche med Wehnschr 32 1368, 1906
- 6 Teague, O., and Torrey, J. P. A Study of Gonococcus by the Method of 'Fixation of Complement,' J med Research 17 223, 1907
- 7 Hollstein, M. Biological Relations of Diplococcus Intracellularis and Gonococcus J Exper Med 9 538, 1907
- 8 Schwartz, H. J., and McNeil, A. The Complement Fixation Test of Gonococcal Infections, J Med Sc 141 193, 1911
- 9 Vannoli, T. Ueber Agglutinine und spezifische Immunkörper im Gonococcusserum Deutsche med Wehnschr 32 1984, 1906
- 10 Krumbein und Shatiloff, P. Untersuchungen über das Meningococcenserum Deutsche med Wehnschr 34 1002, 1908
- 11 Herrold, R. D. Laboratory Methods for Diagnosis of Gonorrhea in the Male J Urol 26 379, 1931
- 12 Jacoby, A. The Unreliability of Laboratory Aids in the Diagnosis of Gonorrhea in Women, Am J Obst & Gynec 23 729, 1932
- 13 Bucher, C. J. When Is Gonorrheal Cure? Pennsylvania med J 35 690, 1932
- 14 Kolmer, J. A. Serum Diagnosis by Complement Fixation, Philadelphia 1928 Lea and Febiger
- 15 Swan, C. S. Results and Interpretation of Complement Fixation Test for Gonorrhea, New England J Med 207 601, 1932
- 16 Barringer, E. D., Strauss, H., and Crowley, D. F. The Problem of "Clinical Gonorrhea" in the Female, Am J Obst & Gynec 25 538, 1933
- 17 Brunet, W. M., and Levine, B. S. A Survey of One Thousand Gonococcus Complement Fixation Tests Performed With Serums of Male Patients in an Outpatient Clinic, Am J Clin Path 3 429, 1933
- 18 Hlem. A Survey of One Thousand Gonococcus Complement Fixation Tests Performed With Serums of Female Patients in an Outpatient Clinic, Am J Obst & Gynec 28 501, 1934
- 19 Cohn, A., and Grafenberg, E. Die Bedeutung der Komplementfixationsmethode für die Diagnose der Gonorrhoe Ztschr f Hyg u Infektionskr 104 128, 1925
- 20 Tulloch, W. J. Serological Examination of 100 Strains of the Gonococcus, Isolated From Cases of Acute and Subacute Urethritis in the Male, J Path & Bact 25 346, 1922
- 21 Cohn, A. Weiterer Beitrag zur Serodiagnose der Gonorrhoe, Med Klin 21 1159, 1925
- 22 Thomson, A. E., Hamann, A. C., and Park, W. H. The Gonococcus Complement Fixation Test, J Immunol 29 249, 1935
- 23 Price, I. N. O. The Clinical Application of the Complement Fixation Test for Gonorrhoea, Brit J Ven Dis 10 249, 1934
- 24 Cohn, A., and Rosowsky, F. Zur Serodiagnose der Kindergonorrhoe, Deutsche med Wehnschr 57 1540, 1931
- 25 Cohn, A. Zur Serodiagnose der Gonorrhoe Deutsche med Wehnschr 52 1717, 1926
- 26 Osmond, T. E. The Value of the Complement Fixation Test in Gonorrhea. A Study of Five Thousand Tests, Brit J Ven Dis 5 281, 1929
- 27 Rubinstein, M., and Gauran, M. Serodiagnostic des affections Gonocoques, Compt rend Soc de biol Paris 89 893, 1933
- 28 Martland, E. M. Complement Fixation Test in Diagnosis of Gonococcal Infection in Women, Brit J Exper Path 4 235, 1923

+++ (positive), ++ or + (weakly positive or doubtful, the weakly positive or doubtful reactions can only be satisfactorily interpreted on the basis of history, clinical and bacteriological findings), - signifies the absence of any complement fixing antibodies

While ++++ and +++ reactions are considered to be indicative of the presence of a gonococcal infection, the weak positive reactions (++ and +) are not evidence of disease if a positive history smear or culture cannot be obtained

## RESULTS

*Comparison of Serologic Results and Clinical Diagnosis*—The material used in the study presented here was obtained in its greatest part, from patients in the New Haven Hospital. A smaller number of serums were furnished through the kindness of Dr. M. J. Strauss from the New Haven Municipal Clinic and other institutions. 1153 tests of 495 patients (470 adults, of whom 199 were males and 271 females and 25 children of whom 24 were females and 1 male) have been performed. Since material for routine diagnosis was taken from individuals who had suspicious clinical signs, but in whom no history or bacteriologic evidence of gonorrhea could be found, there is a large number of negative reactions in this group. On the other hand, patients who had definite bacteriologic gonorrhea were tested in various stages of the disease. Many single serums were examined numerous times in order to study the stability of the reaction in a single specimen. In several instances serum was taken from the same patient at different times in order to determine both the constancy of the reaction and the increase or decrease of complement fixing antibodies. It has been impossible to test some of the serums, because they were either hemolyzed or showed a strong anticomplementary action.

Table I, in which the clinical diagnosis and the serologic findings in each case are presented, demonstrates the relation between the clinical progress of the disease and the increase of complement binding antibodies. The cases are grouped according to sex, where the disease was not systemic in nature. The presence of gonococcal infection in all of the patients was determined bacteriologically. The figures and percentages presented here are not so high as some recorded in the literature, because cases of early and late infections have been included, no attempt was made to determine the maximum reaction in any single case.

Some explanation of the reactions which show a complete absence of complement fixing antibodies seems necessary. It is possible that the antigen used in the test does not contain the strain which has caused infection in any particular negative case. Since 72 per cent of all cases of gonorrhea are the result of infection with a "predominant type," this explanation will hold only rarely. Another possible explanation is that a patient may have lost the power of producing antibodies of any sort (last stage of infection, malignant tumors, etc.). A more frequent reason, however, is that the duration of the infection is too short for antibodies to be produced. As in the Wassermann reaction, the gonococcus complement fixing antibodies are not found before the end of the second or third week. Many investigators believe that they can elicit the reaction

tivity of the differentiation with absolute alcohol or acetone. The selective action of iodized decolorizers is less affected by moisture than that of the usual pure solvents.

It may therefore be recommended to introduce in the old and in the modified Gram's methods one modification more, that is the addition of a little iodine to the decolorizing solvent or mixture. The amount of iodine to be added need not be very exact. It is sufficient to add drops of *Tinctura iodii* to the solvent until it becomes a light brownish color.

The modification is suitable with any one of the well known formulas for gram staining. If acetone or alcohol acetone mixtures are used as decolorizers the iodine must be added at the last moment, because it reacts slowly with acetone if the mixture is stored. It is, therefore, more practicable to choose a staining formula in which absolute alcohol is the decolorizer, since in it the dilute tincture of iodine is stable and can be kept in a drop bottle. Dilute carbol-fuchsin, not safranin, shall be used for the counterstaining, because the yellowish red color of safranin does not contrast as sharply against the greenish black color of the iodine methyl-violet compound, as the more purplish tone of fuchsin.

#### REFERENCES

1. Bemens, T. H. C. Gram Positive and Acid Fast Properties of Bacteria, *J. Path. & Bact.* 17: 199, 1912.
2. Burke, V. New Method for Gram Stain, *J. Bact.* 7: 159, 1922.

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## MEASURING CHANGES IN THE INTRACRANIAL PRESSURE OF EXPERIMENTAL ANIMALS\*

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IT was found necessary to measure changes in cerebrospinal pressure during sacrifice experiments on cats and dogs. Several methods were tried without any degree of success until the following was suggested by Dr. Reginald A. Cutting. Upon trial, it was found to give excellent results.

A steel rod of  $\frac{3}{8}$  inch diameter was turned on the lathe to just the size and shape of the trephine to be used ( $\frac{3}{8}$  inch at the top tapering through  $\frac{3}{4}$  inch to  $\frac{1}{4}$  inch at the cutting edge). Above this was turned a nozzle to fit heavy rubber pressure tubing. A  $\frac{1}{8}$  inch hole was then drilled throughout the length of the rod. The trephine shaped end was threaded with a  $\frac{1}{4}$  20 thread (Fig. 1).

A water manometer was prepared from heavy Pyrex tubing with a  $\frac{1}{8}$  inch bore. The reading column was made 80 cm. and the other column 40 cm. long. The manometer was mounted on a wooden panel which was graduated along the reading column in millimeters. The manometer was equipped with a three way ground glass stopcock in the pressure line leading to the animal (Fig. 2).

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fection remains localized or is cured, no antibody production occurs. In other words, the clinical conclusion that anterior gonorrhea in the male is more easily cured than cervicitis in the female is corroborated by the different percentage of positive reactions obtained with serums taken from patients suffering with these respective types of infection.

The parallelism which exists between serologic and clinical evidence is also seen in cases of posterior urethral gonorrhea. The number of strongly positive reactions is three times as large as in anterior urethral gonorrhea. This increase in antibodies is therefore due to the spread of the infection which, as is known from clinical experience, is frequently followed by other complications such as inflammation of the prostate, seminal vesicles, epididymis or joints. There have been a number of cases of posterior urethritis which showed a negative or only weakly positive reaction. It is possible that the antibodies disappear with treatment or that the infection runs a mild course and consequently no antibodies are produced.

The greater the spread of the infection, the larger is the percentage of positive and strongly positive reactions. Cases of epididymitis, salpingitis, oophoritis, and arthritis show the strongest reaction. It is in these stages of infection that the gonococci usually are not found in the smears made from the discharge. The complement fixation test is the only one which can confirm the clinical suspicions in this stage of the disease. Many investigators put much stress on the association of this clinical picture and positive serologic reaction with coincident negative bacteriologic findings, the intensity of the reaction is thought to be the result of the presence of a closed or latent focus. This may be true in many cases, but the same degree of reaction can be found in patients with apparently open foci. Therefore it seems that it is the virulence of the infecting microorganism together with the resulting response of the infected tissue which produces this strong reaction.

The chronic infection confirms this opinion. Similar pathologic changes, such as the formation of closed or latent foci, may be found. In cases of long standing where the response of the tissue to the invading microorganism is weaker, and the blood and lymph supply in the tissue is less, the absorption of antigen is slow and small in amount. The serologic results may therefore be weakly positive (++) or (+). Even these reactions must be considered seriously in patients with suspicious histories and clinical findings. Repeated tests and clinical and bacteriologic examinations must be performed. An exacerbation of the chronic process will produce an increase of antibodies, while effective treatment may make the reaction negative. Table I, D shows the small number of chronic cases and weakly positive reactions.

In order to check the results reported by Brandstrup, in which he demonstrated that 33 per cent of pregnant patients with positive reactions had a puerperal fever due to the gonococcus, and in order to determine whether there may be nonspecific reactions in serums from such patients, the bloods of 23 pregnant women were tested. Since most of the cases examined showed a negative reaction, it was impossible to confirm Brandstrup's observations. Twenty of this group had no history or findings of gonococcal infection, 18 serums gave a negative reaction, the other two, a weakly positive (+). Two of the 3

In the case of a rise in pressure the zero point reading is subtracted from the actual reading and the result multiplied by two. In a fall of pressure the actual reading is subtracted from the zero point reading and multiplied by two. In either case the answer is in millimeters of water, positive or negative pressure. To obtain the reading in millimeters of mercury the answer must be divided by 13.6

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## THE DISAPPEARANCE OF PHENOLS AND CRESOLS ADDED TO "BIOLOGICAL PRODUCTS" ON STANDING\*

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WITH THE ASSISTANCE OF JOSEPH TRUHLAR AND JULIUS AXELROD

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### INTRODUCTION

"BIOLOGICAL PRODUCTS" may be said to include vaccines, antiserums, and antiplasmas, and various materials such as antitoxin preparations, related to or derived from these. Their importance and significance are increasing rapidly both for therapeutic use and (with perhaps more widespread application) for immunization. Unfortunately, the chemical knowledge underlying the products in question lags far behind their practical applications.

In the preparation of these "biological products," their potencies are necessarily of the highest significance. Their value naturally rests upon the requisite physiologic response of the organism. This phase of the subject does not enter into the present problem. Of perhaps greater importance is the safety factor of these products, that no direct harm will ensue from their use. Many of the products are used intravenously, sub or intracutaneously. All contain protein and, in general, would serve as excellent media for bacterial growths. Such growths may well produce toxins or other harmful products or be themselves a danger if the material containing them were used. One phase of this problem will be taken up here and an experimental investigation of certain actions and relations presented. The results will be limited to vaccines and antitoxin preparations in the present instance, but the methods used and in all probability the conclusions arrived at, possess general applicability.

The safety factor in connection with a bacterial vaccine or an antitoxin material involves its preparation, storage and use. The preparation lies outside this presentation. The product, when ready for use is sterile. To maintain the sterility, a preservative is added. Carbolic acid and the cresols have been perhaps the most commonly used preservatives in amounts up to 0.5 per cent.

In the practical development, the material is prepared preservative added, and sterility insured. It is then put up in vials or other containers of suitable size, and the sterility tested again.

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found to contain material equivalent to 81 per cent calculated as phenol. In other words, a found value of 0.324 per cent calculated as phenol where the cresol mixture was used would correspond to 0.00324/0.81 or 0.4 per cent cresols.

## EXPERIMENTAL RESULTS

The results obtained with vaccines will first be presented and then those with a number of antitoxin preparations which contained much higher percentages of protein (10 to 12 per cent as against 4 per cent or less). With the vaccines, in every case (in Table I) 0.45 per cent phenol was added to the vaccine when it was prepared. A number of vaccines were obtained which had been kept in vials with rubber stoppers at ice box temperatures for different lengths of time up to six years. Because of the small quantities of some of the vaccines which were available, different kinds were combined for a number of tests. Those combined and run together had been prepared at practically the same date. These results are presented in Table I.

TABLE I

PHENOL CONTENTS OF VARIOUS VACCINES (CONTAINING ORIGINALLY 0.45 PER CENT PHENOL) AFTER STANDING AT ICE BOX TEMPERATURES THE INDICATED NUMBER OF DAYS

CHARACTER OF VACCINE	NUMBER OF ORGANISMS PER CC	TIME OF STANDING (DAYS)	PER CENT PHENOL FOUND
Pertussis	5 billion	1845	0.327
Gonococcus	4 billion	1456	0.293
Cholera	8 billion	1820	0.304
Combined streptococcus	12 billion	780	0.331
Typhoid	1 billion	972	0.427
Catarrhalis combined	12 billion	174	0.409
Catarrhalis combined	12 billion	824	0.355
Catarrhalis influenza	14 billion	814	0.277
Catarrhalis influenza	14 billion	1830	0.248
Influenza combined	3 billion	1300	0.101
Pertussis	5 billion	10	0.456
Staphylococcus	2 billion		
Pertussis	5 billion	385	0.380
Staphylococcus	2 billion		
Pertussis	5 billion	732	0.340
Typhoid	1 billion		
Typhoid	1 billion	2205	0.274
Staphylococcus	250 million		
Typhoid	1 billion	1100	0.311
Influenza	750 million		
Gonococcus	1200 million	265	0.417
Acne	440 million		

The results in Table I showed marked decreases in the phenol contents after standing. Unfortunately, in this part of the study, it was not practicable to study the same products for the different periods of time. In view of the preliminary or orienting nature of the tests, it is not advisable to attempt to draw general conclusions from them. It may be stated, however, that the numbers of killed organisms did not appear to be the main factor involved.

It seemed possible that the rubber stoppers which were used with the vials might also play a part perhaps by taking up some of the phenol and removing it from the sphere of action. Experiments were therefore carried out with

# MODIFICATION OF THE GRAM METHOD\*

## USE OF IODIZED DECOLORIZERS

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WHEREAS there is a classical method for the Ziehl-staining, which is everywhere employed, there are over a hundred modifications of the Gram method, none of which is generally recognized as being markedly superior to the others. Such a profusion of prescriptions might be explained if on one point of the original method there was a defect, which has escaped attention in successive modifications.

The mechanism of the gram-staining has been analyzed by many workers, and according to Beniens,<sup>1</sup> the iodine forms with the pararosaniline dyestuffs a complex of high molecular weight, the membrane of the gram-positive bacteria being impermeable to it. Burke<sup>2</sup> has given an experimental confirmation of this hypothesis. But those authors have only considered the diffusion and retention of the iodine complex as if it were thoroughly stable.

But the decolorizer may produce an additional effect. It may dissociate the complex, especially if its action is prolonged. The iodine-methyl-violet-compound appears to be rather loose, and the iodine, which has great affinity for the solvents used as decolorizers, may dissociate and diffuses out progressively, and of course the liberated violet will diffuse likewise.

If this is true, the addition of iodine to the decolorizer will inhibit the dissociation of the iodine dyestuff complex and promote a much more selective differentiation. Experience confirms fully this claim, as is shown by the results in Table I.

TABLE I

TIME OF DECOLORIZATION OF ALL OR ALMOST ALL THE BACTERIAL BODIES OF PURE-CULTURE FILMS

(Method: Violet, 20 sec.; Lugol, 20 sec.; Decolorizer, Alcohol-Acetone 2/1)

	IODIZED DECOLORIZER	DECOLORIZER WITHOUT IODINE
<i>B. coli</i>	5 sec.	5 sec.
<i>Staphylococcus</i>	1 hour	5 minutes

Table I shows that, notwithstanding the iodine content of the decolorizer, the differentiation time for the gram-negative bacteria does not change, while use of such iodized decolorizer increases about ten times the resistance of the dyed gram-positive organisms against the decolorization.

The contrast is still more obvious in the case of smears of organic products of irregular thickness, as sputum or vaginal secretion. It is known that the water retained in the smears from the aqueous stains diminishes the selec-

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The results obtained with some antitoxin solutions are given in Table III. The times of standing refer to the material in vials or syringes. Five and 10 cc containers were used (in a few cases 2 cc and 25 cc). The vials and syringes were stored in the ice box ( $5^{\circ}\text{C}$ ) and tested after the indicated lengths of time. Preparations within the range A to R refer to diphtheria antitoxin, and X to Z to tetanus antitoxin.

The vials were closed with small rubber stoppers, while the syringes in addition to the rubber stoppers contained rubber plungers with considerable surfaces (the width of the containers) exposed to the solutions. The results show that, with phenol in the vials, there was a marked decrease in one case only (Experiment E). The remainder showed no definite decreases. The only experiment with a syringe sample (R) showed a real decrease. In the experiments in which the cresol mixture was used, it must be recalled that 0.4 per cent cresols added correspond to a found value in terms of phenol of 0.324 per cent. The one vial containing cresols (A) showed a value of 0.258 per cent, and the three syringes (N, Q, L) values of 0.173, 0.176 per cent. The lengths of times of standing in all cases where marked decreases were shown were more than a year.

#### DISCUSSION

In the first place, the accuracy of the phenol estimations is sufficiently great to make it possible to draw definite conclusions even for changes considerably less than those presented here. It may be stated that estimations of phenol contents of these products made in a different laboratory agreed surprisingly closely with the results found in this laboratory. The decreases in the phenol contents may therefore be accepted as established.

The question of the safety of these and other products handled similarly must be considered. Just what percentages of phenol (or of cresols where these were used) constitutes "safety" cannot be stated arbitrarily. While 0.5 per cent seems to allow a fairly wide margin, much may depend upon the special organism present. Data on these questions are being collected elsewhere and will be presented in due course.<sup>3</sup>

The fact that the rubber stoppers are the cause of the disappearance of the phenol from the vaccine and the antitoxin preparations raises a number of questions, such as the most satisfactory type of stopper, the possibility of obtaining phenol resistant stoppers, etc., which lie beyond the scope of this paper.

The character of the combination of rubber and phenol was not investigated.

In the practical use of vaccines, antitoxins, etc., it is most desirable, if not essential, to determine the phenol content at definite intervals.

Although the results presented in this paper are limited to vaccines and antitoxins, it is evident that any biological product containing phenol (or cresols) as preservative and stored in rubber stoppered containers, is open to suspicion.

#### SUMMARY

Phenol, used as a preservative in maintaining the sterility of vaccines and antitoxins, was found to decrease in amount when these products were stored in rubber stoppered vials, or in the presence of rubber stoppers. The rubber was found to remove the phenol from the vaccine.

In use the parietal bone of the cranium of the animal is laid bare and a hole drilled with the trephine. This is cleaned thoroughly and an incision is made through the dura. Then with the aid of a pair of pliers the threaded end of the steel plug is screwed tightly into the cranium. With the three-way stopcock completely open, the manometer is connected to the plug with heavy rubber

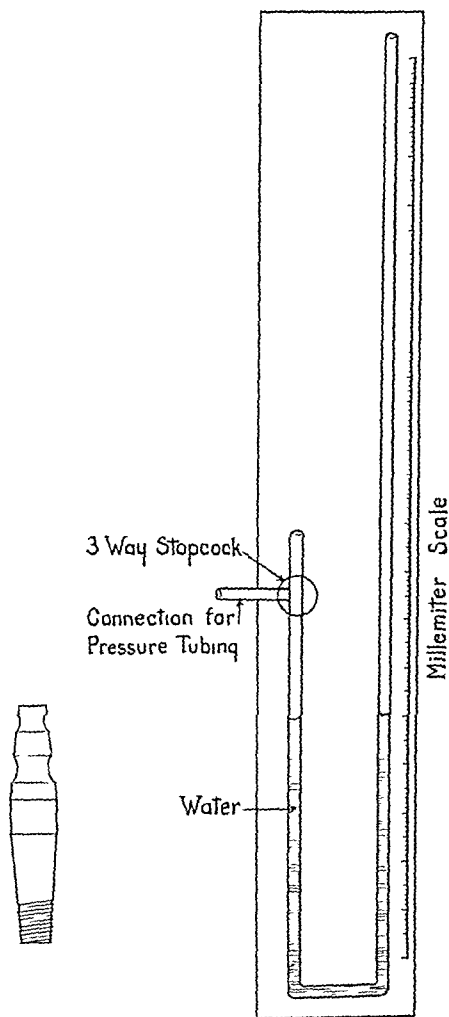


Fig.1  
[Full Size]

Fig.2

pressure tubing. Care must be taken to insure that all joints are absolutely air-tight. The stopcock is then turned through 45 degrees so that there is an open connection only from the plug to the manometer. A reading of the manometer is immediately taken. This represents the zero point or normal for that animal. Readings showing rise or fall in pressure may then be made at any desired interval during the experiment.

containing a 40 per cent formalin solution, the cover slip being attached only on two sides by petrolatum. The trichomonads per cubic millimeter were after some minutes immobilized.

Test Tube 5	9 Trichomonads
Test Tube 6	8 Trichomonads
Test Tube 7	9 Trichomonads

The addition of two drops of 1 per cent formalin to a 5 cc sample of urine, thus to be examined, was found to be simpler and therefore preferable (as similarly experienced in our culture studies) to the exposure of untreated urine to formalin fumes. Later, it was learned that as little as two drops of 0.25 per cent formalin to the urine or culture frequently accomplished the same result.

Finally, Tubes 1, 2, 3, and 4 were centrifuged for five minutes at 3,000 revolutions per minute. Four cubic centimeters of the supernatant fluid was completely removed by a capillary pipette, and the hemocytometer was filled with the mixed residue or sediment. The apparently fixed, hyaline like trichomonads were readily visible and easily counted. No evidence of any destruction of the organisms was encountered. The number of organisms per cubic millimeter of residue of voided urine was as follows:

Test Tube 1	40
Test Tube 2	41
Test Tube 3	49
Test Tube 4	40

These studies are not as extensive as one would wish, but such material as is necessary for further investigation has been encountered rarely. In order to repeat the above experiments in so far as possible, the trichomonads in urine were simulated by the addition of 0.25 cc of a well mixed culture of *Trichomonas hominis* in Andrews' modification of serum saline citrate medium<sup>2</sup> to 4.75 cc of urine, such urine having been previously determined as being normal in all respects. This was incubated overnight. In the morning the trichomonads were found to be actively motile. To each tube of 5 cc of this trichomonad infested urine was added two drops of 1 per cent solution of formalin. The hemocytometer was then filled, and the results were as follows:

Tube 1	First count	31
	Second count, another sample	35
Tube 2	First count	35
	Second count, another sample	26
	Third count, another sample	34

To determine the effect of centrifugalization on trichomonads, this urine was then centrifugalized at about 3,000 revolutions per minute for five minutes. Four cubic centimeters of the supernatant fluid was removed. This contained no demonstrable organisms. The residue and the remaining supernatant fluid were mixed with a capillary pipette and the number of organisms was then determined by the hemocytometer method as indicated, with results as follows:

Tube 1	First count	118
	Second count, another sample	123
Tube 2	First count	141
	Second count, another sample	134

Two factors may now enter into the problem. After a certain length of time, definite for each type of product (expiration date), the vaccine, antitoxin (or other product), is returned to the producer, retested for potency, and if found satisfactory and sterile, revialed and redistributed for use. Also, at times, larger vials may be employed and the material of any one vial may not be completely used immediately but only after an interval, as, for example, when immunizing a larger number of individuals, or when one individual is immunized with the same material at intervals.

In these cases, the possibility of contamination of the material exists. Presumably the preservative present will destroy or kill any accidental bacterial contamination. It was observed, however, that products to which 0.45 per cent phenol had been added originally did not contain this amount of phenol after a time. As this raises an extremely important point in connection with the safety factor, a study of this apparent disappearance was undertaken. A number of the results obtained will be presented.

#### EXPERIMENTAL METHODS

The experimental work to be presented will include the estimation of phenol and cresol in a number of vaccine and antitoxin preparations which had been kept under the customary conditions of storage of biological products.

The significance of the results will depend upon the accuracy of the phenol estimations. The method of analysis consisted in the quantitative formation of tribromophenol by the addition of a bromide-bromate solution and titration of excess bromine with thiosulphate after the addition of potassium iodide, hydrochloric acid, and starch. The method was used first by Koppeschaar,<sup>1</sup> studied further by a number of workers, and a careful reinvestigation given in detail by Scott<sup>2</sup> several years ago.

In view of Scott's extended study and detailed presentation of the method, it will not be repeated here. For the removal of protein, the well-known Folin-Wu precipitation was used. To 5 c.c. of the vaccine or antitoxin, 35 c.c. of water were added, then 5 c.c. of 10 per cent sodium tungstate solution and 5 c.c. of 0.67 N sulphuric acid. The mixture was shaken, allowed to stand at room temperature for thirty minutes, and filtered through paper. To 40 c.c. of the filtrate, 160 c.c. water were added, and the phenol estimation carried out.

Duplicate determinations as a rule agreed within 0.5 per cent of the amount of phenol found. Using the purest phenol obtainable, satisfactory recoveries, within 2 per cent, of the amount added, were obtained.

The results will be given in terms of percentage of phenol present, although in a number of the preparations a bactericidal material containing a mixture of the three cresols was used.

It was shown by Scott that the method of analysis was accurate for the estimation of phenol as stated, but that for the cresols, while the determination could readily be carried out, there was not a quantitative formation of the tribrom derivatives of the ortho and para compounds. Where such a cresol mixture was used in the data to be presented, the results will be calculated and given in terms of phenol. The cresol mixture when analyzed as described was

hemocytometer When the organisms are too active for accurate counting, the chamber containing the material may be exposed to the fumes of formalin by inverting it over the mouth of a bottle containing a 40 per cent solution, the cover slip being attached only on two sides by petrolatum thus fixing the organisms

The ability to centrifuge samples either of urine containing this organism or of the returns of a douche in vaginal trichomoniasis should aid in detecting mild or subsiding involvements which otherwise might appear to be negative In addition, there is nothing in the morphologic and biologic characteristics of trophozoite forms of other flagellates which would prevent the satisfactory application of this method if and when they are detected in the type of material here noted It would seem that the number of vegetative forms of amebae and ciliates, as well as cysts of all protozoa (except for trichomonas cysts of which so far none have been demonstrated) could be counted similarly, but without the exhibition of formalin since the degree of motility of these forms would not interfere with the procedure of counting

Finally, the great dilution of formalin which appears to be toxic to trichomonads suggests its possible use in the therapy of trichomonad infection or infestation, both in the urinary and digestive tracts

#### SUMMARY

1 It is possible now to determine quantitatively the degree of urinary trichomoniasis by the use of the hemocytometer The flagellates are counted as are cells in spinal fluid and the number per cubic millimeter of the sample examined is thus obtained When the protozoan is too motile for accurate enumeration, two drops either of 0.25 per cent formalin or of 1 per cent formalin are added to 5 c.c. of the material Centrifugalization without apparent injury to the trichomonads is possible, especially if formalin is used as indicated prior to this procedure

2 The application of this method in vaginal trichomoniasis, as well as a modification of it outlined in the text for the numerical determination of these organisms in prostatic secretion, has been suggested

3 The clinical significance of this procedure rests in the ability to estimate quantitatively the extent of infestation, its progression or decline, and the efficacy of therapy

4 There is nothing in the morphologic and biologic characteristics of other flagellates, amebae and ciliates, if and when so encountered, that would prevent a satisfactory application of this method

5 The great dilution of formalin which appears to be toxic to trichomonads suggests its possible use in therapy, both in the urinary and digestive tracts

#### REFERENCES

- 1 Paulson, M., with the assistance of Morgenstern, M. An Accurate Method for the Numerical Determination of *Endamoeba histolytica* In Vitro and Its Possible Use With Other Intestinal Protozoa, Suggested Clinical Application, Am J Trop Med 12 387, 1932

influenza and typhoid vaccines, at 3 to 5° C. and 37 to 38° C., and with rubber-stoppered and glass-stoppered containers. The results of a series of tests with these two vaccines, each containing 2 billion killed organisms per c.c., and in the presence and absence of rubber stoppers, are presented in Table II. The amount of phenol added was 0.5 per cent, and each vaccine was divided into 3 portions of over 500 c.c. each. One portion (A) was placed in a glass-stoppered bottle after the addition of a number of rubber stoppers to the liquid and kept at 37° C. for 237 days and at 3 to 5° C. for twenty-two days, a second portion (B) was placed in a glass-stoppered bottle and kept at 37° C. for 237 days and at 3 to 5° C. for twenty-two days, and a third portion (C) was placed in a glass-stoppered bottle and kept at 3 to 5° C. for 259 days. The results of the phenol determinations at the end of the time are given in Table II.

These results indicate unmistakably that the rubber stoppers caused the decreases in the phenol contents, that the character of the organism and the temperature of storage played an insignificant or no part in these changes.

TABLE II

PHENOL CONTENTS OF INFLUENZA AND TYPHOID VACCINES (CONTAINING ORIGINALLY 0.50 PER CENT PHENOL) AFTER STANDING IN GLASS-STOPPERED BOTTLES UNDER THE INDICATED CONDITIONS

TREATMENT	TIME AND TEMPERATURE OF STANDING	PER CENT PHENOL FOUND
<i>Influenza Vaccine:</i>		
A. Rubber stoppers present	237 days 37° } then 22 days 3-5° }	0.390
B. No rubber stoppers	237 days 37° } then 22 days 3-5° }	0.517
C. No rubber stoppers	259 days 3-5°	0.501
<i>Typhoid Vaccine:</i>		
A. Rubber stoppers present	237 days 37° } 22 days 3-5° }	0.389
B. No rubber stoppers	237 days 37° } 22 days 3-5° }	0.511
C. No rubber stoppers	259 days 3-5°	0.497

TABLE III

ANTISEPTIC CONTENTS IN TERMS OF PERCENTAGES OF PHENOL FOUND IN ANTITOXIN PREPARATIONS AFTER STANDING VARIOUS LENGTHS OF TIME

PREPARATION	TYPE OF CONTAINER	PER CENT ADDED		STANDING (DAYS)	FOUND, CALCULATED AS PER CENT PHENOL
		(RESOLS)	PHENOL		
C	Vials	0	0.5	0 (Bulk)	0.460
G	Vials	0	0.5	0 (Bulk)	0.511
F	Vials	0	0.5	334	0.513
E	Vials	0	0.5	340	0.380
A	Vials	0.4	0	1040	0.258
R	Syringes	0	0.5	376	0.325
N	Syringes	0.4	0	457 } mixed 1036 }	0.173
Q	Syringes	0.4	0	775	0.176
L	Syringes	0.4	0	1426	0.174
Z	Vials	0	0.5	77	0.509
X	Vials	0	0.5	475	0.503
Y	Vials	0	0.5	544	0.468

TABLE I  
STEPS OF FUNGUS

	MEDIUM USED	ACTINOMYCETES (AEROBIC STAIN)	ACTINOMYCETES (ANAEROBIC STAIN)	COCCIDIODES	EIDERMOUTHYTON	NOVILLA
pH 7.3	3-2% HEAT INFUSION BROTH					
	1% Dextrose		Growth at bottom of tube	Luxuriant growth		Luxuriant growth
	1% Dextrose layer of mineral oil					
	1% Dextrose saturating cotton surmount ed by potato slant	Luxuriant growth		Luxuriant growth	Moderate growth	
	0.1% Dextrose slant 0.5% Dextrose slant 1.0% Dextrose slant Stab	Good growth Good growth Good growth		Good growth Good growth Luxuriant growth	Good growth Good growth Luxuriant growth	Good growth Good growth Luxuriant growth
pH 6.7	Solidified agar 2% (hormone media may not be filtered)	Good growth Good growth Good growth	Growth on surface	Good growth Good growth Luxuriant growth	Good growth Good growth Luxuriant growth	Good growth Good growth Good growth
	0.1% Dextrose slant 0.5% Dextrose slant 1.0% Dextrose slant	Good growth Good growth Good growth				
	The anaerobic strain of actinomyces, very difficult of culture died out before completion of the experiment. Stab cultures were attempted but not obtained upon the various sugar contents.					

## REFERENCES

1. Koppeschaar, W. F.: Maassanalytische Bestimmung des Phenols, Ztschr. f. anal. Chem. 15: 233, 1876.
2. Scott, R. D.: Application of a Bromine Method in Determination of Phenol and Cresols, Indust. & Engin. Chem. Anal. Ed. 3: 67, 1931.
3. Falk, C. R., and Aplington, S. P.: Studies on the Bactericidal Action of Phenol and Merthiolate Alone and in Mixtures, Am. J. Hyg. 24: 285, 1936.

## THE NUMERICAL DETERMINATION OF TRICHOMONAS HOMINIS IN URINE AND ITS PRACTICAL IMPLICATIONS IN GENITOURINARY PARASITISM\*

MOSES PAULSON, M.D., BALTIMORE, Md.

RECENTLY, in demonstrating a clinical problem to students, a voided specimen of urine from a male was found to contain trichomonads, presumably *Trichomonas vaginalis* Donn , 1837. In discussing the degree of involvement it was pointed out that this, probably, would have to be determined upon mere impression gained from the microscopic examination of varying sized droplets. However, the knowledge derived in the establishing of a method for the numerical determination of protozoa in vitro, which was modified to apply clinically in two cases of human intestinal parasitic involvement,<sup>1</sup> was used to count the trichomonads present in this voided urine as follows:

Seven tubes, each containing 5 c.c. of the well-mixed specimen, were treated in the identical manner as were *Trichomonas hominis* cultures in the study referred to above, except that 1 per cent formalin, found to serve the same purpose just as effectively, was used instead of 10 per cent. The experiences with this urine appeared to be identical with those of the cultural studies. The organisms were found, as occasionally occurred in our cultures, to be too motile for accurate hemocytometric counting; then a drop or two of 1 per cent formalin placed in the urine resulted in nonmotile, apparently fixed, hyaline-like and, as far as could be determined, nondisintegrated trichomonads. The organisms were counted by the hemocytometer as are cells in spinal fluid. This method of counting needs no further description here. The high dry power lens and a mechanical stage were used in these determinations, thereby facilitating the procedure and increasing accuracy.

The number of trichomonads per cubic millimeter of voided urine was as follows:

Test Tube 1	8 Trichomonads
Test Tube 2	9 Trichomonads
Test Tube 3	9 Trichomonads
Test Tube 4	10 Trichomonads

Next, the hemocytometer containing other samples of this urine untreated with formalin was inverted for a few minutes over the open mouth of a bottle

\*From the Departments of Medicine, Gastro-Intestinal Clinic (Dr. T. R. Brown, chief), and Pathology and Bacteriology of the Johns Hopkins University and Hospital.

Received for publication, June 18, 1936.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFF, M.D., ABSTRACT EDITOR

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## **MENINGOCOCCUS MENINGITIS, The Control of Epidemics by Active Immunization With Meningococcus Soluble Toxin, Kuhns, D. M. J A M A 107 5, 1936**

An epidemic of meningococcal meningitis occurred in which there were seventeen cases

a The greatest number of cases occurred where there was the greatest density of population, in a CCC camp near a small town

b The mortality in the epidemic was 83 per cent in six untreated patients outside the CCC camp and 11 per cent in nine patients treated early with antimeningococcus serum

A culture for carriers was made in the CCC camp, Camp Crystal, on four successive weeks and the highest number found positive at one time was 44 per cent. A culture was made at the same time on a nonepidemic camp 20 miles distant and 35 per cent were positive

On another cultural study made by Ross Laybourn at Camp Coffey, 35 per cent were found positive.

It was noted that cases continued to occur in the CCC camps after all the control measures available at the present time were enforced. The control measure considered to be of the greatest value was the separation of individuals the greatest distance possible from one another.

A study of filter separable fractions of meningococcus broth culture was made to determine the skin reactive properties of Gordon's four types of meningococcus

A It was found that the whole culture produced the greatest reaction and the filtrate produced the next greatest reaction

B The heated filtrate produced no reaction and served as a control

C. The filtrate from autolyzed meningococci, or endotoxin, produced no skin reaction.

The filtrate was given a trial in an epidemic of meningitis to determine its value as a skin testing and immunizing substance

A new approach as to how to determine the level of immunity of a susceptible individual by skin testing with graduated dilutions of filtrate on an individual coming down with the disease and again after recovery is suggested as a possible method of determining the skin test unit dose

a The amount of filtrate necessary to change the skin test from positive to negative should be the amount necessary to immunize the individual

b. The skin test reaction could be a very valuable guide in the treatment of the individual.

The filtrate was used in a dilution of 1:100 as a skin testing substance on two and four week convalescents and gave consistently negative tests

Skin testing on sensitive and susceptible individuals is the only available means at the present time of determining the toxicity of the exotoxin. Although the laboratory animals do react, the effects are not sufficiently consistent to titrate the potency of the product.

A 1:100 dilution of a filtrate produced from Types 1, 2, 3, and 4 meningococcus was used to make skin tests of 2,000 newly enrolled CCC members; 37 per cent gave a skin reaction of one plus or greater.

The 4 c.c. of supernatant fluid which was removed from each of these tubes was then replaced, the material mixed thoroughly and the counts of the two tubes made as follows:

Tube 1:	29
Tube 2:	36

This was similarly repeated with identical methods, using another strain of *Trichomonas hominis* at a later date, with the following results:

Tube A:	First count	50
	Second count, another sample	46
Tube B:	First count	35
	Second count, second sample	38

After centrifugalization and removing 4 c.c. of the supernatant fluid, as noted, the remaining material, mixed thoroughly, gave these counts:

Tube A:	First count	262
	Second count, second sample	252
Tube B:	First count	221
	Second count, second sample	220

When the 4 c.c. of the supernatant fluid which had been drawn after centrifugalization was replaced in its respective tube, and then well mixed with the residue, the following counts were obtained:

Tube A:	54
Tube B:	47

#### COMMENT

The closeness of these counts is obvious; hence the relative accuracy of the hemocytometer for practical purposes in the counting of trichomonads in urine needs no further comment. Incidentally, the coefficient of variability in the use of the hemocytometer in the numerical determinations of *E. histolytica* in vitro was found by me to be under 5 per cent.<sup>1</sup> It is interesting to note that Hegner<sup>3</sup> and his students first used the hemocytometer in trichomonad studies in lower animals. As far as can be determined, this communication is its first recorded clinical application.

Our studies indicate that trichomonads in urine, treated with formalin, are not destroyed when submitted subsequently to centrifugalization. Identical results from a limited number of *Trichomonas hominis* serum-saline-citrate cultures when submitted to this identical procedure would tend further to confirm this.

It seems possible to determine quantitatively the degree of urinary trichomoniasis by the numerical determination of the protozoan in the manner described above. Thus, progression of the condition and the efficacy of therapy can better be evaluated. No cases of vaginal trichomoniasis have been available for investigation by this method. However, it is suggested that a quantitative determination could be made by treating the returns of a douche of a pint or quart of saline in the same manner as noted for trichomonads encountered in urine. Trichomonads in prostatic secretion can also be determined by the

The corpus luteum cells of the ovary are likewise characterized by a very low nuclear nucleolar ratio

The study of the nucleoli is emphasized as valuable help in the tumor diagnosis, although none of the changes reported represents a criterion absolutely characteristic for each malignant cell

Because of lack of knowledge concerning the biological functions of the nucleolus, the authors can, at the present time, offer no interpretation of their observation

#### LEAD POISONING, Ratio of Large to Small Lymphocytes in, Shiels, D O Med J Australia 1 847, 1936

Absorption of lead causes an increase in the ratio of large lymphocytes plus monocytes to small lymphocytes

A fall in this ratio below 2:1 while the subject is exposed to the hazard is associated with definite symptoms of lead poisoning, usually of sufficiently severe nature to cause incapacity

Generally speaking, the more severe the case, the lower the ratio

The magnitude of this ratio is more closely associated with the clinical condition than is the stippled cell count

The magnitude of this ratio is a simple and very useful indication by which to judge of the imminence or otherwise of lead poisoning and is an aid to diagnosis

#### LYMPHOGRANULOMA, Use of Mouse Brain Antigens for Diagnosis of, Grace, A W, and Suskind F H. Arch Dermat & Syph 34 65, 1936

It has been found that Frei antigen can be prepared from the brain of a mouse infected with the virus of lymphogranuloma inguinale

The original Frei antigen is prepared from pus from a human being has many drawbacks

There are at least four distinct conditions due to lymphogranuloma inguinale which may be diagnosed by the performance of the Frei test

Antigen prepared from mouse brain has been tried for one year at the New York Hospital for the diagnosis of lymphogranuloma inguinale The preparation and administration of 88 different specimens of antigen derived from the brains of mice used for the second to the forty fourth passage of a single strain of virus and the results of their use in 27 patients with the disease and in 38 nonlymphogranulomatous subjects are described in detail

The average positive reaction to antigen prepared from mouse brain was an erythematous papule 8.7 mm in diameter, with a surrounding flare of varying size 77 per cent of these reactions showed a central papule from 7 to 10 mm in diameter

No reaction which could be regarded as positive was obtained in any person by the use of antigen prepared from lymphogranulomatous mouse brain in nonlymphogranulomatous persons or by the use of emulsions of normal mouse brain prepared in the same way as the antigen

The results of tests on patients with conditions which must be differentiated from lymphogranuloma inguinale were definitely negative

The presence of active or arrested syphilis had no effect on the reaction to the Frei test performed with antigen prepared from mouse brain

By standardization of dilution and dosage it was possible to produce reactions quantitatively the same as those to antigens prepared from human pus, qualitatively, however, the reactions were usually more intense

No desensitization of the skin of any lymphogranulomatous subject was observed after repeated inoculations with antigen prepared from mouse brain Low grade sensitization to the protein of mouse brain, however was seen in one instance

Antigen prepared from mouse brain has been found to retain its potency for at least eight months after preparation

2. Andrews, J. M.: Cultivation of Trichomonads; Thermal Death Point; Anaerobic Conditions; Attempts at Sterilization, *Parasitology* 12: 148, 1926.
3. Hegner, R.: Differential Reactions of Species and Strains of Trichomonad Flagellates to Changes in the Environment, *Am. J. Hyg.* 16: 513, 1932.
4. Ratcliffe, H. L.: Studies on Trichomonads in Rats, *Am. J. Hyg.* 8: 901, 1928.

MEDICAL ARTS BUILDING

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## HORMONE MEDIA AS A BASIS FOR FUNGUS CULTURES\*

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MARTHA VAIL, B.S., LOS ANGELES, CALIF.

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AFTER having accidentally obtained several fungus growths upon routine blood hormone agar slants from unsuspected cases, we began to experiment with hormone media, basing our efforts upon the premise that the beef heart hormones facilitated the growth of fungi just as they did that of the bacteria.

This work has been carried on as a part of the routine of the laboratory, covering a period of six months; hence, the variety of cultures used has been limited necessarily to those obtained from patients in the hospital during that time. By using the beef heart infusion medium, we have been able to uncover more fungus infections than we had succeeded in doing previously in an equal period.

In our experiments, the basis of the media used was heart infusion broth, made after a modified formula of Huntoon. This was used in several variations as seen in Table I.

Since Sabouraud's medium is slightly acid, pH 5.7, the hormone medium was reduced from its natural alkalinity of pH 7.3 to pH 5.7, and parallel cultures of several strains of fungi (actinomyces, coccidioides, monilia, epidermophyton) were inoculated upon slants having the two reactions. No perceptible difference in rapidity or volume of growth could be noted.

In obtaining material from the patients it was found advisable to eliminate contaminating bacteria as far as possible by the thorough sterilization of the field surrounding the lesion and observing the most careful technic, as even the acid version of this medium does not inhibit bacterial growth as does Sabouraud's.

The highest percentage of positives was obtained when several types of the media were inoculated, and incubation was continued for several weeks. One strain of actinomyces showed a definite preference for the potato slant. A beautiful play of colors was obtained upon this culture; from the original white the whole slant became a deep indigo in two weeks, and later fringes of coral began to appear. Another strain of actinomyces grew only in the broth made anaerobic with mineral oil, and in deep agar stabs, 3 cm. from the surface. A characteristic black growth began to appear in three days. The potato slant and broth proved quite unsatisfactory, however, for an epidermophyton obtained

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# ENDOCRINOLOGY

*The Bulletin of the Association  
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## INTERNAL SECRETIONS

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from a so-called "athlete's foot," but a luxuriant growth appeared after three weeks on the agar slants. The monilia thrived impartially upon all the aerobic types. Several excellent cultures of coccidioides were obtained upon agar slants, although they grew equally well in broth. These develop rapidly even on the blood slants used routinely for bacterial cultures, a white mycelial growth being observed in two or three days, which rapidly becomes luxuriant a few days after transplanting to the dextrose slants.

#### CONCLUSIONS

The use of beef heart infusion as a basis for culture media for fungi is highly satisfactory. Since modifying the pH does not inhibit the bacterial growth, and the fungi grow luxuriantly in the alkaline medium, and since a 1 per cent dextrose seems to be slightly more favorable to growth than a lesser amount, it is concluded that this percentage in the natural alkaline medium is the simplest and most satisfactory to use.

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Although the filtrate that was used in the Tarkio epidemic did not appear as stable as the one the authors are developing at the present time, the skin test there indicates a trend from positive to negative

A filtrate aged 9 months in a refrigerator held at 50° C reacts on 10 per cent less of the same enrollees than one made nine months later

An opportunity to observe the change in skin tests after subcutaneous inoculation with full strength filtrate, after an interval of four months, was offered at Camp Coffey and the trend was definitely from positive to negative.

Among 395 individuals receiving meningococcus filtrate subcutaneously, none have developed meningitis, even though in the Tarkio epidemic four cases developed among those not immunized. Ten months have intervened since this immunization was first initiated

The filtrate has been used in three outbreaks not reported in this paper with the same results as described

It is to be remembered that this is only a preliminary report and in order to prove the value of the meningococcus filtrate as a skin testing and immunizing agent it should be used in a large number of beginning epidemics and over a long period of time

#### RENAL FUNCTION The Excretion of Ferrocyanide in Man in Relation to the Urea Clearance, Gordon, W. Am J M Sc 192 208, 1936

The ferrocyanide excretion test of renal function was compared with the simultaneous urea clearance in 102 subjects with a range of clearance levels from 6 to 130 per cent. Following intravenous injection of 0.28 gm of sodium ferrocyanide, normal subjects excreted at least 24 per cent in one hour, 35 per cent in two hours, and 50 per cent in three hours. There was a correlation of about 0.7 between urea clearance and ferrocyanide excretion for each of these three periods. One hour proved to be an adequate length of time for the test. The administration of ferrocyanide did not alter the clearance. In those instances in which the tests gave discordant results other evidence pointed to error in each of the two methods with about equal frequency. The physiologic significance of the test is discussed. Considerable value in detection of impairment of renal function may be expected of the ferrocyanide test, but its value in observing the progress of renal disease cannot be judged yet and is somewhat more uncertain. The test is simple and harmless in most instances. Great care is required in estimating ferrocyanide in the presence of hematuria and some accuracy is sacrificed if much blood is present in the urine. Patients with prostatic hypertrophy had dysuria and inability to void in some instances

#### UNDULANT FEVER, Treatment of, by the Intravenous Injection of Killed Typhoid, Paratyphoid A and Paratyphoid B Bacilli, Ervin, C E, Hunt, H F, and Niles, J S Am J M Sc 192. 234, 1936

Of 12 cases of undulant fever observed, 10 were treated by the intravenous administration of typhoid vaccine. The results in all 10 were satisfactory. There have been no recurrences and the follow up agglutination tests for *Br. abortus* were negative in 9 of the 10 patients treated. Two follow up agglutinations in control cases were also negative

Those results compare favorably with the treatment reported by other authors who have used specific substances prepared from *Br. abortus*

The distinct advantages of typhoid vaccine are its availability in all localities and the simplicity with which it can be administered

#### TUMORS, Cytological Studies of Malignant, Von Haam, E, and Alexander, H. G. Am J Clin. Path 6 394, 1936

By means of a modified biometric method, the nuclear nucleolar ratio was determined in 10,000 cells chosen at random from fifty malignant tumors and fifty benign tissues.

A comparison of average values, obtained from groups of one hundred cells examined in each specimen, demonstrates that 96 per cent of the malignant lesions showed a nuclear nucleolar ratio distinctly lower than that found in the benign tissues



# Peptones for Bacteriological Culture Media

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Proteose-Peptone is recommended as a nutriment for the pathogenic bacteria. The higher forms of nitrogen present in this peptone make it exceptionally valuable for the more fastidious organisms. The longevity of the strains is increased and their virulence is maintained for longer periods of time in media prepared with Proteose-Peptone.

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Bacto-Tryptone is an excellent peptone for use in the detection of indol production by bacteria. Reliably accurate results are obtained in relatively short periods of incubation when Bacto-Tryptone is employed for this test. Bacto-Tryptone is also an excellent nutriment for many bacteria, and is particularly recommended for use in media for the cultivation of lactobacilli.

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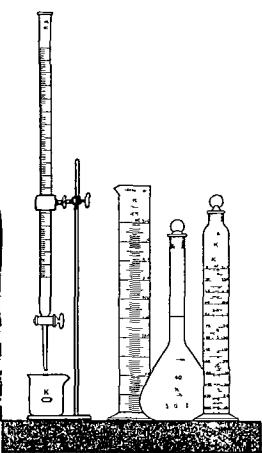
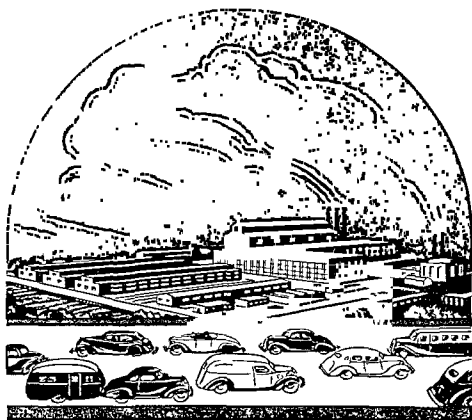
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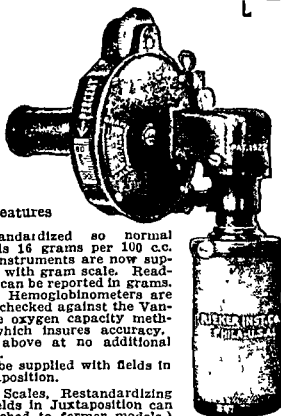
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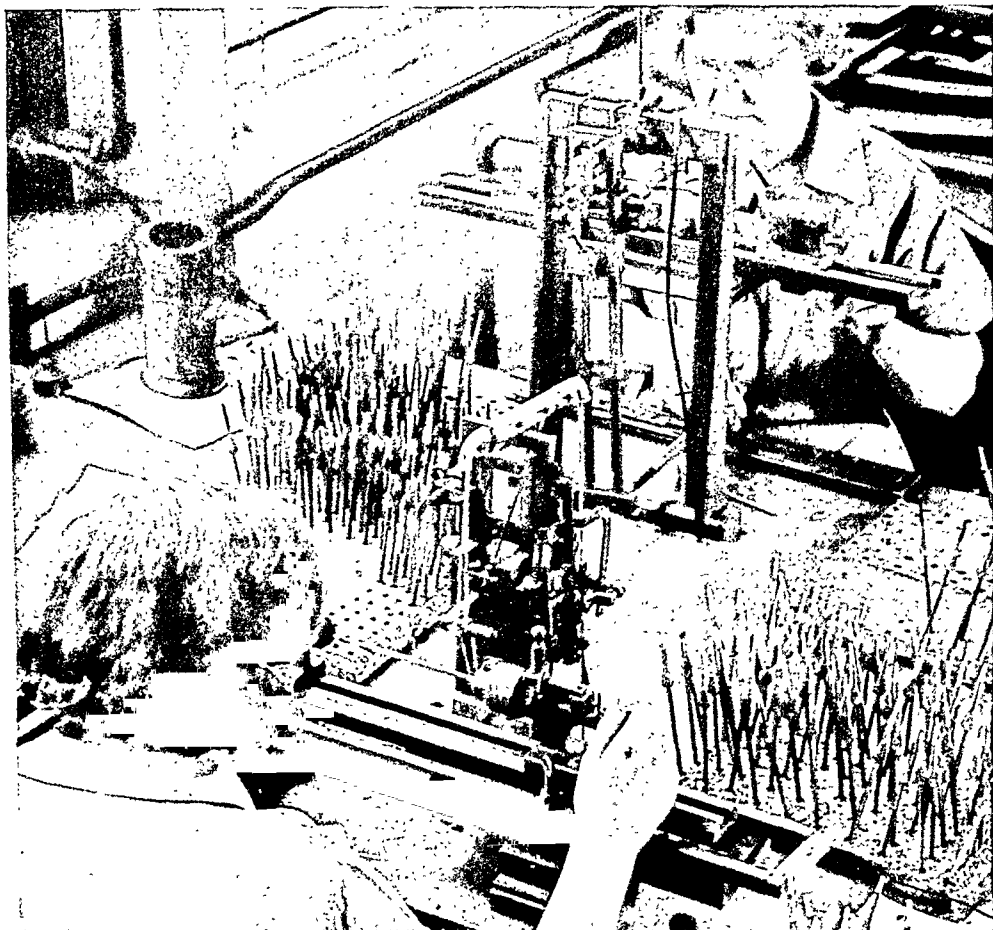
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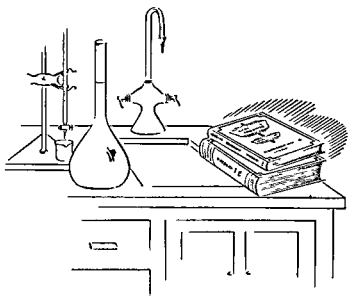
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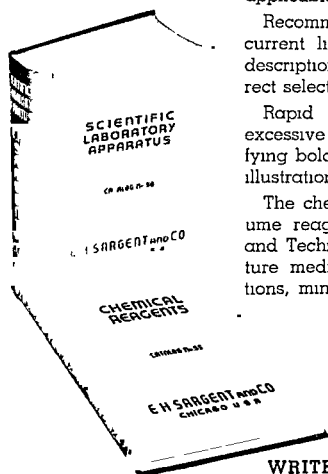
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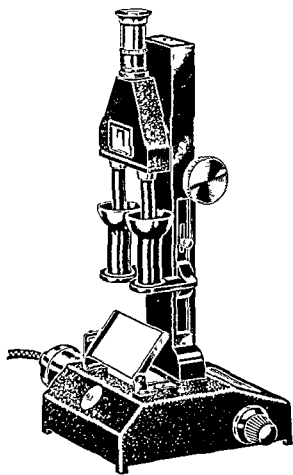
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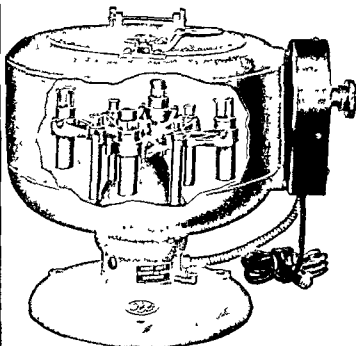
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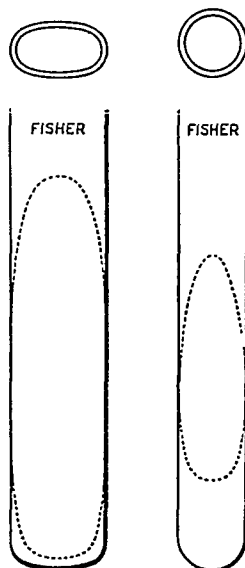
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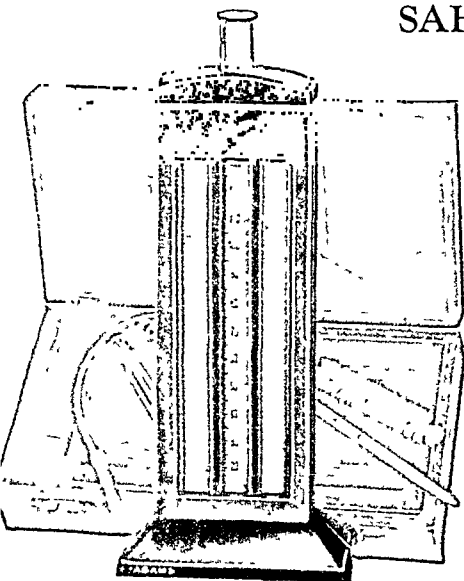


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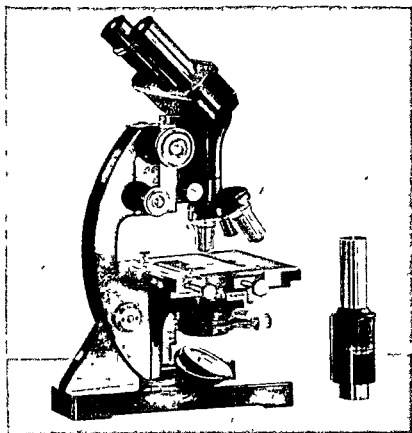
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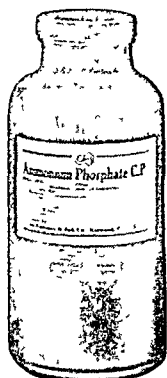
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# *The Journal of Laboratory and Clinical Medicine*

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VOL 22

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No 8

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## CLINICAL AND EXPERIMENTAL

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### FURTHER STUDIES ON THE MECHANISM OF DIURESIS WITH ESPECIAL REFERENCE TO THE ACTION OF SOME NEWER DIURETICS\*

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GEORGE HERRMANN, M D, AND GEORGE M DECHERD, JR, M D, GALVESTON, TEX  
WITH THE ASSISTANCE OF PETER S ERHARD, CLARENCE C PEARSON,  
R C DOUGLAS, MISS ELSIE ROBERTS AND OTHERS

---

THERE are still sharp differences of opinion as to where and how diuretic drugs act. American investigators for the most part are agreed that the kidney is the site of the chief and preponderant pharmacologic effect. The continentals, particularly German investigators, insist upon the presence of extrarenal factors in the production of diuresis. Some postulate a "physico-therapeutic water freeing" effect upon the tissue colloids with a resulting temporary hydiemia. This increase in blood volume by dilating the renal vascular bed is considered by Meyer<sup>1</sup> to account for the actual outpouring of urine. Jackson<sup>2</sup> demonstrated some pharmacodynamic effect of mersalyl on most, if not all, of the vascular bed of the dog. He pointed to a possible analogy to A N Richards'<sup>3</sup> findings of a quantitatively different action of substances, which had been found to cause less contraction of the afferent than of the efferent arterioles to and from the glomerular tufts, thus leading to increased urine formation in Bowman's capsules. These theories do not admit of clinical investigative approach.

#### THE BLOOD SERUM PROTEIN STUDIES

Shally,<sup>4</sup> after some confirmatory physicochemical studies, accepted the conclusion of Nonnebruch and others that changes in the colloid osmotic pressure

---

\*From the Negro Medical Service of the John Sealy Hospital and the Department of the Practice of Medicine, University of Texas Medical Branch.

Received for publication June 1 1936

Presented before the Section on Pharmacology and Therapeutics A.M.A. May 12 1936, at Kansas City

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merisalyl The blood Na remained fixed, but the urinary Na conspicuously increased but usually not in the tremendous proportions of the Cl excretion, so that the ratios Na/Cl as a rule decreased Occasionally, however, the reverse was noted and the Na/Cl ratio increased In these studies of ours, neither the mercurials nor the xanthines showed consistent or characteristic effects on the Na/Cl ratio

If the volume of urine, with its contained sodium and chloride, passed during the control hours, be subtracted from the corresponding figures after injection of the diuretic, we obtain a fair approximation of the excess of these substances mobilized from the tissues during diuresis By assuming the edema fluid to be an ultrafiltrate of blood plasma, we can calculate the excess amount of sodium and chloride that should be excreted, with the excess urine excretion of the same composition as the tissue fluid The values for sodium calculated on this basis show a good agreement with those actually found, while the actual excretion of chloride is in most instances greatly in excess of the calculated values

#### CREATININE CLEARANCE STUDIES

Four years ago we reported our studies<sup>13</sup> anent the mechanism by which diuresis is accomplished following the intravenous injection of theophylline, as aminophyllin and organic mercurial salts, as saliyan and combinations of the two in patients with congestive heart failure and edema We employed the creatinine clearance method as described by Rehberg<sup>14</sup> to measure the glomerular filtration, and we found in our edematous cardiacs, as Schmitz<sup>15</sup> did in the creatinine clearance experiments in the dog, that the xanthines or purines, theophylline ethylene diamine (theophylline, metaphyllin or aminophyllin), greatly augmented the glomerular filtration, while the mercurial, merisalyl, apparently produced a diuresis by inhibiting tubular reabsorption

Our clinical experiences along with our experimental theoretical considerations convinced us that there was a very definite complementary action of the xanthines and mercurials when administered in close succession or simultaneously The clinical results seemed further to support our conception of the mechanism of diuresis and the different points of action of the two types of drugs We reported further studies using dextrose as well as creatinine clearance as a measure of glomerular filtration and secured confirmatory evidence of our thesis Sucrose intravenously was found to have in itself too powerful a diuretic action White and Monaghan<sup>16</sup> had found the creatinine clearance as good a test as any of glomerular function, and in spite of Shannon's<sup>17</sup> and Fulton's<sup>18</sup> condemnation, we consider that the creatinine clearance in comparative studies, such as we have been doing, has proved satisfactory

A translation has recently been furnished us of the paper written in Hungarian by Issekutz and Vegh<sup>19</sup> on experimental studies of diuretics on the toxicity and action of various organic mercury compounds, prepared by Foldy and his associates of the Chmion laboratory In studying the toxicity of the complex sodium acetate salts of mercury, they added a slightly acid theophyllin solution to reduce the strongly alkaline reaction that caused so much pain on

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WARREN T. VAUGHAN, M.D., Editor  
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in 1 c.c. of the 10 per cent solution 0.0396 gm. Hg. Thus the actual difference in mercury content between mersalyl and mercurpurin is negligible. Mercurpurin differs chemically from mersalyl only in having camphoric acid substituted for the salicylic acid, and theophyllin added. This substitution is said to have made the preparation about half as toxic as mersalyl. The new compound was called for the sake of brevity "novurit" in Europe and in this coun-

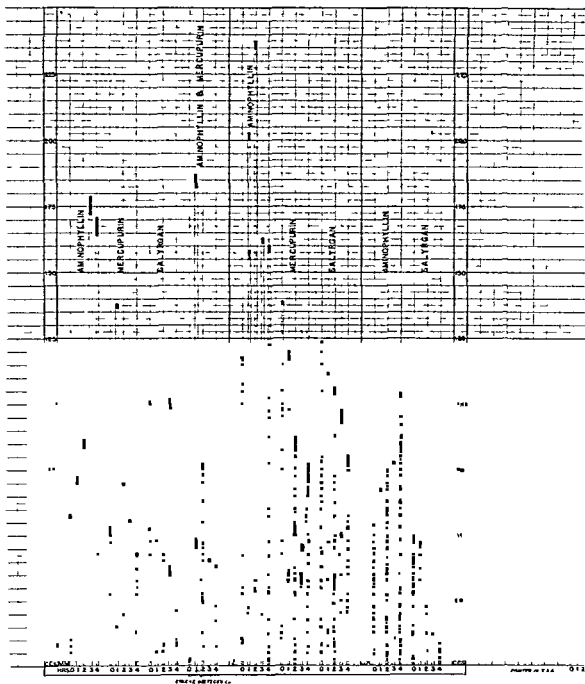


Fig. 2.—Charts on other patients made up just as those in Fig 1 showing the increased filtration as a rule following aminophyllin intravenously and decreased reabsorption diuresis after mersalyl injection and combination effects with the mercurpurin administration

try "mercurpurin." The mercurate camphoric acid without the theophyllin added has been offered as mercurial diuretic suppository "mercurin" for rectal administration.

#### PRESENT STUDIES ON THE MECHANISM OF DIURESIS

Introduction of the new compound that is a combination of the mercurial and xanthines with reputed superior diuretic properties seemed to confirm our earlier contentions of the different mechanism of action for each of the two

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larily in the relief of persistent epinephrine fast asthmatic states. In some 50 asthmatic patients, intravenous aminophyllin can be shown to result in prompt increase in the vital capacity and blood flow through the lungs. Mercurials do not produce such spectacular and prompt respiratory relief, but after profuse mercurial diuresis, dyspnea is usually less paroxysmal attacks subside.

#### THE RESULTS

The present creatinine clearance studies confirmed and supplemented our previous observations. A survey of the data revealed the fact that in the twenty five studies on the seven edematous patients it seemed to make little difference which drug was introduced first. Of course, the interval of three to five or more days allowed the effect to be dissipated before the second drug was used. In all experiments satisfactory diuresis was accomplished.

The 7 experiments in which aminophyllin from an ampule of 2 cc containing 0.48 gm diluted 10 times with saline was used intravenously presented further conclusive evidence of a conspicuous increase in glomerular filtration in every instance. In the 7 experiments in which mersalyl, 2 cc of a 10 per cent solution, was introduced, the creatinine clearance studies showed consistently little if any rise and usually a gradual decrease in the filtration as the diuresis increased. In other words there was, along with the increasing defect in reabsorption, a slight decrease in filtration. In four instances in which 2 cc of 13.5 per cent solution of mercuprim with 3.5 per cent combined, 1.5 per cent free theophyllin was used, the creatinine clearance showed a predominance of the mercurial effect in two of the cases, that is, there was no rise in filtration but decreased reabsorption, while the xanthine effect, an increased filtration, predominated in three instances. The increase in filtration was a very definite, though often somewhat delayed, occurrence. In three instances in which mercuprim was fortified with aminophyllin there was often a very temporary decrease in the filtration before diuresis began but this was followed by a conspicuous rise in the glomerular filtration along with the characteristic mercurial impairment of tubular absorption. This fortified combination produced a more striking diuresis.

Blumgart, Gilligan, Levy, Brown and Volk<sup>21</sup> were not able to demonstrate any theophyllin augmentation of filtration in six studies on three normal individuals who were under very carefully controlled conditions.

In our previous and present creatinine clearance studies in edematous patients, we have demonstrated a total of 17 experiments in which theophyllin preparations were used, with a very definite and often conspicuous increase in glomerular filtration. In 3 instances in which aminophyllin was combined with mercuprim, a similar evidence of definite increase in filtration was obtained and likewise in 3 out of the 5 cases in which mercuprim alone was used, a transient increase in filtration was demonstrated. In the 17 experiments in which mersalyl was used and the creatinine clearance studies carried out, a defect in reabsorption was the striking finding in every instance. In the 7 mersalyl experiments of the present series and in 2 of 5 mercuprim experi-

did not explain the movements of water in diuresis. However, he found an absolute and relative increase in the albumin fraction with lowered and unchanged globulin and a drop in fibrinogen at the onset of diuresis in all cases. He tried the effect of mersalyl on blood serum in vitro and concluded that in the concentration that it appeared in the blood and after injection it produces no change in the serum protein, even though at higher concentration there was flocculation. However, in this country, Bieter and Wright<sup>5</sup> have demonstrated with their ultramicroscope changes in the blood serum of those who had received therapeutic doses of the mersalyl. Such ultramicroscopic alterations, we believe, could change the "salting out" levels of the blood protein constituents and account for Shally's shifts in the ratios. The changes in blood protein values that he reported seem to us not to be beyond the limits of the accuracy of the method.

We have studied the blood proteins in ten diuretic experiments, in which we have used aminophyllin, mercupurin and mersalyl and have not been able to confirm Shally's findings in regard to shifts in the blood proteins. We have also determined the dried weight of the serum and have not been able to demonstrate any significant or consistent changes that would indicate an effective hydremia.

#### THE SODIUM AND CHLORIDE RATIOS

Hitzenberger and Engelmann<sup>6</sup> found that the excretion of many inorganic and organic substances, but particularly NaCl, was augmented by mersalyl. Simmert<sup>7</sup> reported similar increases in the urinary NaCl, determined as Cl, with concomitant rises in the blood plasma NaCl, also determined as Cl, occurring in diuresis along with decrease in the hemoglobin and red blood cells. He concluded that his findings indicated a mobilization of the tissue NaCl and a flow of the tissue edema fluid into the blood. Bua<sup>8</sup> on the basis of studies of the effect of mersalyl in diabetes insipidus concluded that the presence of the chloride ion was the *sine qua non* requirement for the diuretic action of mercury salts. Weingarten<sup>9</sup> of Volhard's school has held, as indisputable, the hydrophigenous property of the chloride ion.

Presser and Stahl,<sup>10</sup> however, incriminated the Na ion as the edema producer which effect could be compensated for by a balanced combination with others, as K, Ca, and Mg ions. Bruman and Jenny<sup>11</sup> found a parallelism between sodium excretion and the volume of the urine.

Siedek and Zuckerkandl<sup>12</sup> found more retention of Na than of Cl, therefore  $\text{Na/Cl} > 1$  in pleural and ascitic effusions and in the edema fluid of congestive cardiac failure. Upon the application of diuretics a marked excess of Na over Cl appeared in the urine.

#### OUR SODIUM AND CHLORIDE DATA

In our attempt to elicit the initiating factors and those at work during the first five to six hours of diuresis, we determined the urinary Na/Cl ratios before, hourly during, and as a follow-up, each twenty-four hours for several days after each of our 25 diuretic experiments with aminophyllin, mercupurin, and

parative nature both with mercurpurin and mersalyl intravenously and with mercurin administered as suppositories. They found the newer preparations to be rather more efficient.

The most complete clinical studies of mercurpurin were made by DeGriaff, Nadler, and Batterman. Their 20 edematous patients were observed under carefully controlled conditions and the body weight fluctuations taken as the evidence of effectiveness of diuretic agent. Besides studying the effect of mersalyl and mercurpurin alternately in the same patient, these investigators secured a preparation containing only the organic mercurial or mercurpurin without the theophyllin. Contrast of the effect of this pure mercurial preparation used intravenously and under the same conditions with the effects of the combination, mercurial + xanthine, preparations showed that the theophyllin added definitely to the effectiveness of mercurpurin. They had no general toxic mercurial and no local reactions from intravenous administration of nearly 100 injections. They concluded that mercurpurin was a safe diuretic as well as an effective one and that the combination preparation induces a greater diuresis than mercurials alone and that the superiority of the xanthine mercurial is chiefly if not entirely due to the presence of the theophyllin.

#### OUR CLINICAL STUDIES

Comparative clinical studies of mercurpurin diuresis as compared with mersalyl diuresis have been carried out in the wards of the John Sealy Hospital for the past two years, and during the past year mercurin suppositories containing mercuramphyl, a mercurial salt similar to mersalyl with camphoric acid instead of salicylic and without theophyllin and put up in a cocoa butter, have been under clinical investigation. Our data concern results in 70 cases of patients with edema who have been observed under standard conditions of a preliminary three to five day rest in bed, on a constant intake of 1,600 cc of fluid and a low protein salt poor diet. Only 5 of these patients suffered primarily from the effects of cirrhosis of the liver, the other patients presented evidences of organic heart disease of one type or another with congestive failure in various stages and of varying degrees.

In these 70 patients, 200 injections of mercurpurin were given intravenously and alternately, for comparison, 115 injections of mersalyl were given in 2 cc doses of each, and 64 mercuramphyl (mercurin) suppositories were administered. The order of the use of the drugs varied from time to time and the initial drug used was alternated. Sometimes the preliminary administration of acid salts simultaneously administered and other times omitted was employed and definite augmentation was always noted no matter what preparation was used. In 25 of the suppository cases for experiment, the preparatory enema was omitted and the results were so poor that they should not be included in the average, and in the other half of the cases an enema was given.

The diuretic results of all of these injections and suppositories were tabulated, the percentage of increase in urinary output during the first and second and third twenty-four-hour periods following the administration of the drug as contrasted to the control daily outputs were calculated and summarized for

administration of the mercurial. Theophyllin not only produced the neutral reaction but also definitely increased the diuretic action of the mercurial compound in experimental animals.

A new preparation described by the distributors as a sodium salt of camphoric acid, allylamine-methoxy-mercury acetate with theophyllin 3.5 per cent combined, 1.5 per cent free, containing 41.1 per cent mercury, but according

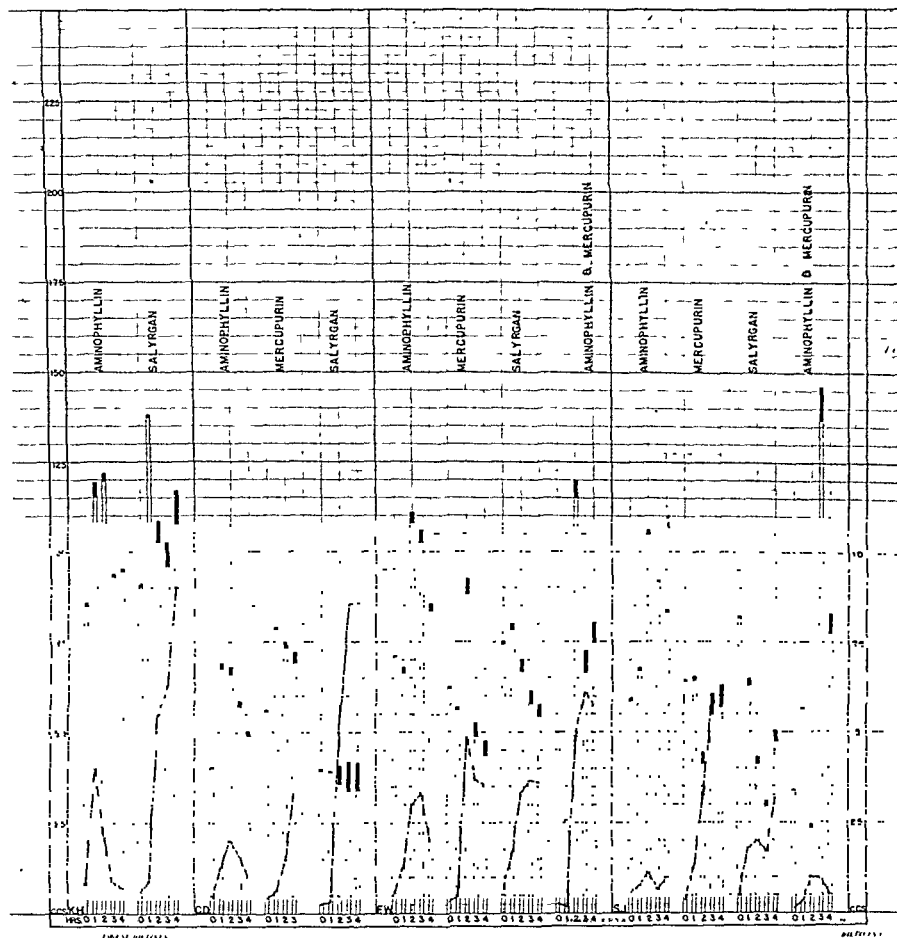


Fig. 1.—Chart showing by hourly columns the height of filtration (top of each column) and the height of reabsorption (bottom of black cap) in cubic centimeters per minute as determined by the creatinine clearance method of Rehberg. The height of the black cap and the rise of the solid line running through the hourly columns indicate the grade of diuresis. Each group of columns represents an experiment following the introduction of one of the diuretics. The experiments on each individual are grouped between the bordering lines and the diuretic used in each experiment is printed above the columns.

to Issekutz and Vegh as containing 38.8 per cent mercury all of which is combined, was introduced into clinical therapeutics. DeGraff, Nadler, and Batterman<sup>20</sup> recently stated that the 13.5 per cent aqueous solution of mercupurin with theophyllin contains 29.1 per cent mercury by weight, so that 1 c.c. contains 0.0393 gm. Hg while mersalyl with 39.5 per cent Hg by weight contains

ANURIA as a result of glomerular tubular destruction which we have noted in two instances following the use of other mercurials intravenously was not encountered in this small series. Mercurin suppositories produced renal stimulation in one instance. It must, therefore, be remembered that the suppository contains 0.5 gm (75 gr.) of mercury.

#### COMMENTS

The results bear out the prediction and the clinical experiences of our earlier studies<sup>12</sup> in which we pointed out the augmentary effect of a combination of theophyllin and the mercurials.<sup>13</sup> Our clinical findings with mercurin further confirm those of other investigators. It is evident that the combination preparation of xanthine and mercurial has advantages in the matter of diuretic efficiency.

Our repeated carefully controlled and comparative creatinine clearance studies in seven patients of this series corroborate our previous contentions, namely, that xanthine preparations, particularly theophyllin or aminophyllin, definitely increase the minute glomerular filtration, while the pure mercurial, as mersalyl, acts quite otherwise in most instances, actually decreasing the glomerular filtration and producing a diuresis primarily by inhibiting tubular reabsorption.

The mercurin creatinine clearance experiments, five in number, carried out in the same way on the same patients, sometimes preceding those with the other preparations and sometimes between them and sometimes afterward, showed in 2 instances clearance figures more like those of mercurial action, namely, decrease in tubular reabsorption, while in the 3 others the xanthine effect of increased glomerular filtration was also temporarily evident.

The creatinine clearance studies in three instances of mercurin fortified with 0.024 gm of aminophyllin showed the most striking combination effect of distinctly increased filtration as well as decreased reabsorption. These experiments in confirming all of our previous studies furthermore seem to establish definitely that the theophyllin does not merely serve to neutralize the excess alkalinity of the mercurial salt but seems to augment the diuresis. The experiment with mercurin fortified by additional theophyllin suggests that more of the xanthine could be added to the mercurin to advantage. The theophyllin simultaneously used or ammonium chloride or nitrate by mouth along with the pure mercurial suppository would be rational therapeutics.

#### CONCLUSIONS

1 We believe that further evidence is added that diuretics, both the xanthine and mercurial, act directly upon the kidney.

2 It is apparent from these creatinine clearance studies that the two types of diuretics act somewhat differently and on different parts of the renal unit. The xanthines affect primarily the glomerular tuft of the unit and by increasing the flow of blood increase filtration, while the mercurial apparently acts by changing the tubular epithelium, so that it is less permeable to the filtrate of water and salts and thus by decreasing reabsorption accomplishes increased urinary output.

types of diuretics and to lend support to our observations of the complementary action of the two drugs. The claims for the new preparation revived our interest in the subject of the mechanism of diuresis, and we repeated our diuretic studies in edematous heart failure cases following the intravenous injection of mercupurin as well as mersalyl and aminophyllin.

#### METHOD

The patients were kept at absolute rest in bed on low protein (50 gm.), salt poor (2 gr.), low fat (115 gm.), and high carbohydrate (200 gm.), total calories 2,035, diet and fluid allowance of 1,600 c.c. a day and no drugs other than morphine for the first five days. During this time the renal function studies were done, the blood and urine chemistry examinations were made and accurate charting of the daily twenty-four-hour intake and output of the fluid and body weight of the patient was begun. All twenty-four-hour specimens of urine were sent to our laboratory for complete study.

On the morning of the sixth day the patient was given 10 gm. of creatinine in 100 c.c. of water at 6 A.M. At 7 A.M. a catheter was introduced and the bladder was completely emptied and the urine collected; a control blood specimen was taken at the same time. At 8 A.M. the first-hour urine specimen was removed by catheter and the blood specimen collected. The second control blood and catheterized urine specimens were taken at 9 A.M. and the diuretic aminophyllin in a dose of 0.48 gm. in 10 c.c. of saline, or 2 c.c. of a 10 per cent solution of mersalyl, or 2 c.c. of a 13.5 per cent solution of mercupurin, or the latter fortified with 0.24 gm. of aminophyllin was injected intravenously. After this the blood and catheterized urine specimens were taken every hour for four hours. Increase in urinary flow was consistently observed in the second half hour after injection of mersalyl and in the second hour after the injection of aminophyllin.

Seven patients, with congestive heart failure of varying etiology in each case, were selected and subjected to twenty-five carefully controlled creatinine clearance diuretic studies.

The theophyllin preparation, aminophyllin, was usually given in the first experiment. Occasionally, however, the mercurial, mersalyl, diuretic was administered first. Usually three to five or more days later, or when the body weight returned to the previous level and the urinary output was low and fluctuated very little, the second preparation, the one that had not been used previously, or mercupurin, the new preparation, was introduced intravenously as the diuretic drug. After another interval of at least three days, the one of the three preparations that had not previously been used was administered and its effects were noted. In three instances the mercupurin was fortified with an additional 0.24 gm. in 1 c.c. of aminophyllin and then injected and the more spectacular results were observed and studied.

Evidence of conspicuous smooth muscle relaxing effects of aminophyllin has been repeatedly noted clinically by us in the prompt subsidence of Cheyne-Stokes breathing, the control of paroxysmal nocturnal dyspnea, and particu-

- Schwab, E. H., Herrmann, G., and Stone, C. T.: The Complementary Action of Certain Antiedemic Drugs, Texas State J. Med. 29: 240, 1933.
- Stone, C. T., Herrmann, G., and Schwab, E. H.: The Treatment of Edema in Congestive Heart Failure, South. M. J. 27: 113, 1934.
14. Rehberg, P. B.: Studies on Kidney Function. I. The Rate of Filtration and Reabsorption in the Human Kidney, Biochem. J. 20: 447, 1926.
15. Schmitz, H. L.: Studies on the Action of Diuretics. 1. The Effect of Euphyllin and Salyrin Upon Glomerular Filtration and Tubular Reabsorption, J. Clin. Investigation 11: 1075, 1932.
16. White, H. L., and Monaghan, B.: A Comparison of the Clearances of Creatinine and of Various Sugars, Am. J. Physiol. 106: 10, 1933.
17. Shannon, J. A.: The Renal Excretion of Creatinine in Man, J. Clin. Investigation 14: 403, 1935.
18. Davenport, L. F., Fulton, M. N., van Auken, H. A., and Parsons, R. H.: Creatinine Clearance as Measure of Glomerular Filtration in Dogs, With Particular Reference to Effect of Diuretic Drugs, Am. J. Physiol. 108: 99, 1934.
19. Issekutz, B., and Vegh, F.: A szerves hujanyvegyuletek diuretikus hatasrol, Orvosi hetil. 45: 1928.
20. DeGraft, A. C., Nadler, J. E., and Batterman, R. C.: A Study of the Diuretic Effect of Mercupurin in Man, Am. J. M. Sc. 194: 520, 1936.
21. Blumgart, H. L., Gilligan, D. R., Levy, R. C., Brown, M. G., and Volk, M. C.: Action of Diuretic Drugs. I. Action of Diuretics in Normal Persons, Arch. Int. Med. 54: 40, 1934.
22. Hahn, A.: Novurit, ein neues Diuretikum, Wien. klin. Wchnschr. 42: 1477, 1929.
- Popper, L.: Ein neues Quecksilber—Diuretikum, Novurit, Med. Klin. 25: 912, 1929.
- Popper, L.: Further Experiences With the Mercurial Diuretic, Novurit, Med. Klin. 26: 1229, 1930.
- Saxl, P.: Fortschritte der Diurese-therapie, Wien. klin. Wchnschr. 43: 916, 1930.
23. Crawford, J. H., and McDaniel, W. S.: Some Observations on Mercurial Diuretics, Ann. Int. Med. 8: 1263, 1935.
24. Steuer, L. G., and Wolpaw, S. E.: The Diuretic Action of Mercupurin, J. Lab. & Clin. Med. 21: 298, 1935.
25. Parkinson, J., and Thomson, W. A. R.: A Mercurial (Mercurin) Suppository as a Diuretic for Cardiac Edema, Lancet 1: 10, 1936.

ments, there were not significant increases in glomerular filtration; in fact, there was an actual decrease in filtration as well as a conspicuous decrease in reabsorption that accounted for the diuretic effect.

#### NEW PREPARATIONS

The creatinine clearance studies following the introduction intravenously of the new preparations are indirectly confirmatory. In comparative studies in the same individuals, glomerular filtration figures midway between those obtained with mersalyl alone on the one hand and those following aminophyllin alone on the other hand, have been repeatedly obtained. In only one mercupurin experiment was the increase in filtration at all conspicuous, while 2 of the other 4 showed slight rises in filtration. The inhibitive mercurial effect on reabsorption was, of course, always noted. When the mercupurin was fortified with 1 c.c. of aminophyllin, an unexplained very temporary decrease in filtration was regularly recorded before clinical diuresis had started. This was noted in 2 of 3 instances but all showed within the next hour a very striking increase in filtration as well as the defective reabsorption.

These data not only supported the conception of a difference of the renal site or mode of action of diuretics, but also supported our contention of a dual action of the combined mercurial salt and theophyllin. The experiments seem to furnish evidence that the theophyllin constituent of the new preparation does more than merely neutralize the excessive alkalinity of the mercury salt and actually in these acute experiments it seemed to augment the diuretic property of the latter. The more striking diuresis that resulted from the mercupurin fortified with the additional theophyllin in the form of 0.24 gm. in 1 c.c. of aminophyllin suggests that more theophyllin might be added to mercupurin to advantage.

#### THE NEWER DIURETIC COMPOUNDS IN CLINICAL PRACTICE

This combination of organic mercurials and theophyllin was introduced into clinical practice in Continental Europe as "novurit," and at least a dozen clinical reports bear witness to its efficiency and its nontoxicity. Hahn,<sup>22</sup> Popper,<sup>22</sup> and Saxl<sup>22</sup> are among those who have reported favorably on the preparation. In this country Crawford and McDaniel<sup>23</sup> studied the effect of mercupurin in 15 patients, 10 with edema of heart failure, and 5 with ascites of hepatic cirrhosis, all in advanced stages after long periods of invalidism. Their results were uniformly very satisfactory and the 20 injections of mersalyl used as control gave definitely less diuretic response than did the 118 injections of mercupurin. Studies of the postdiuretic urine specimens showed no renal irritation, and blood urea levels did not rise after the injection of either preparation. No untoward reactions from the injections of either drug were encountered. Steuer and Wolpaw<sup>24</sup> reported a very small series of 35 injections of mercupurin with no control of mersalyl injections and came to conclusions which added nothing to those previously made on more substantial bases. In England, Parkinson and Thomson<sup>25</sup> have conducted studies of a clinical com-

2 Probable Error of the Mean (P E m) =  $6745 \times (\sigma \sqrt{N})$

3 Significance Value (S) =  $\frac{\text{Difference of Means}}{\sigma \text{ d of Diff}}$

4 Standard Deviation of a Difference Between Means  
 $(\sigma \text{ d}) = \sqrt{\left(\frac{\sigma_1^2}{N_1}\right) + \left(\frac{\sigma_2^2}{N_2}\right)}$

d, Algebraic difference between arithmetical mean and a single observation

d<sup>2</sup>, Summation of differences<sup>2</sup> for a series

N, Number of determinations

# FINDINGS

Fig 1 shows a frequency distribution curve for the free and total cholesterol concentrations. From this curve may be seen the complete range of findings, which are 32.1 to 83.7 mg per cent and 98.1 to 300.0 mg per cent for free and total cholesterol, respectively. The total range of the per cent of free in total cholesterol is from 24.4 to 38.6 per cent.

Applying the statistical formulas noted above, the mean values and standard deviations for each of the three groups are obtained (Table I). The mean for the total cholesterol is 160.8 mg per cent  $\pm$  38.7 mg per cent (S.D.). This indicates that at least two thirds of all values here fall between 122.1 mg per cent and 195.5 mg per cent. In like fashion the mean for the free cholesterol

TABLE I  
FREE, TOTAL, AND PERCENTAGE OF FREE IN TOTAL CHOLESTEROL VALUES FOR  
PEPTIC ULCER PATIENTS

TOTAL CHOLESTEROL	COMPLETE RANGE OF VALUES IN MG %	ARITH MEAN MG %	STANDARD DEVIATION ( $\sigma$ )	PROBABLE ERROR OF MEAN	SIGNIFICANT VALUE (S) COMPARED WITH NORMAL
	98.1 — 300.0	160.8	38.7	3.4	8.4
Free cholesterol	32.1 — 83.7	47.7	11.1	0.97	
Per cent free in total cholesterol	26.3% — 34.5%	30.4	4.1%	0.35	

determinations is 47.7 mg per cent  $\pm$  11.1 mg per cent (S.D.) and that for percentage of free cholesterol is 30.4 per cent  $\pm$  4.1 per cent (S.D.).

In comparing the findings of our peptic ulcer series with normal values, it was deemed most consistent to employ the findings of W. M. Sperry,<sup>4</sup> who kindly made available his figures before actual publication, inasmuch as the basic procedure for both groups of determinations was that of Schoenheimer and Sperry.<sup>1</sup> In a series of 126 determinations on normal adults, male and female, he obtained a mean total cholesterol concentration of 209.8 mg per cent  $\pm$  48.6 mg per cent. There was no significant difference obtained between the pre and postabsorptive divisions of this series, remarkable constancy being maintained for the percentage free cholesterol, the mean value ascertained as 26.9 per cent  $\pm$  1.4 per cent. Fig 2 is a frequency distribution curve for the total cholesterol concentrations obtained by Sperry, the complete range being from 131.5 mg per cent to 392.0 mg per cent.

The most outstanding difference between our series and such a group of normals is a definite shift to the left (lower values) of the frequency distribu-

us by C. C. Pearson, a senior, and R. C. Douglas, a junior medical student, employed on NYA. funds. Our clinical results are given in Table I.

TABLE I  
CLINICAL SUMMARY OF DIURETIC EFFECTS

NO. OF INJECTIONS	DRUG	AVERAGE INCREASE IN OUTPUT OF URINE			
		1ST 24 HR.	2ND 24 HR.	3RD 24 HR.	TOTAL
200	Mercupurin	430%	92%	91%	613%
115	Mersalyl	293	92	42	427
25	Mercurin suppositories without enema	92	41	95	228
70	Mercurin suppositories with enema	264	66	25	355

Taking all of the results as they come, the conditions of course varying, now in favor of one drug and now in favor of the other, seemed to favor mercupurin. The average responses for the first, second, and third days following administration of mercupurin showed increases of 430 per cent, 92 per cent, and 91 per cent, respectively, a total of 613 per cent over the urine output of the day preceding the injections; while for mersalyl the averages during the same period showed 293 per cent, 92 per cent, and 42 per cent, a total of 427 per cent increase for the three days over the control day. A secondary rise after the first day was occasionally noted following mercupurin. The level of the control day was, of course, established at a fairly fixed level by the conditions of the experiment, namely, the three- to five-day rest preceding the administration. The table gives a summary of the clinical diuretic effects and also shows that both mercurial diuretics accomplish maximal effect within the first day; both drop about equally during the second day; but there was often persistence of the effects during the third day after mercupurin, while the mersalyl diuretic effect dropped considerably on the third day.

The mercurin suppositories showed a 264 per cent increase for the first day, 66 per cent during the second, and 25 per cent during the third day, a total of 355 per cent following their use; a definitely better effect, in fact, a threefold augmentation of urinary output when the enema was used to prepare the bowel. The diuretic effects did not persist as actively as after intravenous use, showing 67 per cent on the second day and 73 per cent on the third, a total of 370 per cent. Preliminary oral administration of xanthines and of acid salts likewise distinctly augmented the diuresis that resulted from mercurin suppositories.

In only one instance have we had any evidence of renal irritation, and in this case the irritation was first noted after the introduction of mersalyl but did not clear up under continued use of mercupurin. The albuminuria, however, cleared after a rest period, and the subsequent administration of mercupurin caused a prompt exacerbation of the albuminuria; nitrogen retention, on the other hand, actually decreased under the continued use of mercupurin, even though the albuminuria persisted. No other albuminurias nor any instances of intolerance or toxicity of mercupurin were encountered in our series; in fact, in cases in which stomatitis and colitis had been previously produced by other mercurials, mercupurin was tolerated without such reactions.

tion curve for total cholesterol, as may be seen from Figs. 1 and 2. The significance value (*S*) is obtained between our mean of 160.8 mg per cent  $\pm$  38.7 mg per cent and Sperry's normal mean of 209.8 mg per cent  $\pm$  48.6 mg per cent, and this is found to be 8.4, thereby establishing the trend toward a hypocholesteremia for our series of patients with peptic ulcer. The other observation of importance is the generally higher percentage free cholesterol found in the peptic ulcer patients. Although not marked, this change from the normal suggests that the fall in total cholesterol concentrations is due in greater measure to the decrease of ester cholesterol rather than free (or uncombined) cholesterol.

It is of interest to note that the range of values for our peptic ulcer series, duplicates in extent, even though shifted to slightly lower values, the findings in the series of so called normal individuals. We have been unable to find factors in individual cases which would consistently account for the values obtained therein. Particular attention was paid to the question of anemia, but no correlation could be obtained between cholesterol concentration and level of erythrocytes or hemoglobin. Table II gives a brief summary of findings in each case used for this study.

#### DISCUSSION

The true significance of the tendency to hypocholesteremia in patients with peptic ulcer cannot be stated without further investigation. Cantarow,<sup>5</sup> in his review of cholesterol knowledge, finds that hypocholesteremia occurs in hyperthyroidism, acute infections, pernicious and aplastic anemias, hemolytic jaundice, tuberculosis, widespread liver damage, prostatic and intestinal obstruction. Essentially, these conclusions are concurred with by Stone<sup>6</sup> in his review. It is beyond the scope of this paper to assign any of the above factors as the etiologic factors in peptic ulcer, on the basis of our cholesterol findings. It is of interest to note, however, that Sperry<sup>4</sup> found an elevated percentage free cholesterol only in cases of infection or liver damage, and that we<sup>7</sup> demonstrated a hypocholesteremia in acute and subacute stages of infection (primary and secondary luetics). Stoesser<sup>8</sup> has likewise found a comparable hypocholesteremia in acute infections with an elevation of the percentage free cholesterol at the height of the infection.

The hypocholesteremic trend in this series of patients with peptic ulcer is doubly significant, since almost all of these individuals had been on a relatively high fat diet for varying periods of time. One cannot but feel that the tendency to lowered blood cholesterol values here argues against the cholesterol level standing in relationship to exogenous food metabolism. Agreeing with this point of view is a partially completed series of determinations on medical students in which we<sup>9</sup> found no significant variations in either free or total serum cholesterol. The work of Blotner<sup>10</sup> and Boyd<sup>11</sup> is in accord with these findings. In contrast to the above, however, it is only fair to point out the studies of Okey and Stewart<sup>12</sup> and the review of Cantarow<sup>5</sup> who feel that, while there are no true diurnal cholesterol fluctuations, the blood levels will be higher in individuals on a high fat diet for long periods of time.

3. The combination of xanthine with mercurial seems to be logical on theoretical grounds, as we have previously pointed out. The fact that the theophyllin with its mild acidity helps to neutralize the excessive alkalinity of the mercurial salt is another advantage that makes for safety and ease of tolerance.

4. The clinical experiments seem to corroborate the theoretical considerations, in that the combination preparations have definite advantages in efficiency when contrasted to the mercurials in pure form and over aminophyllin when introduced alone.

5. The mercury suppository, mercurin, constitutes an innovation that has many advantages, even though the results are not as spectacular as those following intravenous injections of mercupurin or mersalyl; nevertheless, they are quite striking and gratifying. Even if the preparation were only half as active, it would still have a place for itself by virtue of the ease of administration, and the comparative safety for the rectal administration in contrast to its intravenous medication. In fact, the latter mode of therapeutic approach is never entirely without danger, and whenever effective, other routes of administration should be chosen. This is particularly so for the man in the field, the general practitioner, who is not regularly doing intravenous work. It is quite an extra job to prepare and transport sterile equipment necessary for intravenous injections.

#### REFERENCES

1. Meyer, H. H., and Gottlieb, R.: *Die Experimentelle Pharmakologie*, Berlin, 1933, Urban and Schwarzenberg, 489.
2. Jackson, D. E.: *The Pharmacologic Action of Mercury in Organic Combination*, J. Pharmacol. & Exper. Therap. 29: 471, 1926.
3. Richards, A. N.: *The Nature and Mode of Regulation of Glomerular Function*, Am. J. M. Sc. 170: 781, 1925.
4. Shally, H. O.: *Veränderungen der Bluteiweisskörper bei der Salyrgandiurese*, Deutsches Arch. f. klin. Med. 177: 368, 1934.
5. Bieter, R. N., and Wright, H. N. G.: *Fundamental Studies on the Pharmacology of Mercury Diuretics. In The Kidney in Health and Disease*, Berglund, et al., Philadelphia, 1935, Lea & Febiger, p. 701.
6. Hitzengerber, K., and Engelmann, F. L.: *Ueber das spezifische Gewicht des Harnes bei Salyrgandiurese*, Ztschr. f. klin. Med. 129: 290, 1935.
7. Simmert, H. U.: *Über das diuretische wirkung organischer Quecksilberverbindungen*, Klin. Wchnschr. 14: 530, 1935.
8. Bua, F.: *Über die Wirkungsart der Quecksilberdiurese bzw. anti-diurese*, Klin. Wchnschr. 14: 934, 1935.
9. Weingarten, R.: *Über Kochsalzersatzmittel*, München. med. Wchnschr. 79: 965, 1932 I.
10. Presser, H., and Stahl, R.: *Untersuchungen ueber hydropigene Ioninwirkungen auf lebende Gewebe mittels Durchströmung in Tierversuch*, München. med. Wchnschr. 80: 559, 1933 I.
11. Bruman, F., and Jenny, F.: *Der Einfluss der Ernährung auf den Stoffunisatz bei der Arbeit*, Deutsches Arch. f. klin. Med. 177: 527, 1934.
12. Siedek, H., and Zuckerkandl, F.: *Die Bedeutung des Natrium—Chlorquotienten im Harn beim "Volhardschen" Wasserversuch*, Klin. Wchnschr. 14: 1428, 1935.
13. Herrmann, G., Stone, C. T., and Schwab, E. H.: *Some Studies in the Mechanism of Diuresis in Patients With Congestive Heart Failure*, Tr. A. Am. Physicians 47: 279, 1932.
- Herrmann, G., Stone, C. T., Schwab, E. H., and Bondurant, W. W.: *Diuresis in Patients With Congestive Heart Failure*, J. A. M. A. 99: 1647, 1932.
- Herrmann, G., Schwab, E. H., and Stone, C. T.: *Further Studies on the Mechanism of Diuresis in Patients With Congestive Heart Failure*, Tr. A. Am. Physicians 48: 364, 1933.
- Herrmann, G., Schwab, E. H., Stone, C. T., and Marr, W. L.: *On the Advantage of Alternating the Vegetable and Metallic Diuretics in the Treatment of Edema of Congestive Heart Failure*, J. LAB. & CLIN. MED. 18: 902, 1933.

TABLE II—CONT'D

NO	AGE	NUTRI TION	DURATION SYMPTOMS	ANEMIA	ADDITIONAL REMARKS	CHOLESTEROL		
						TOTAL MG %	FREE MG %	% FREE
51	47	Poor	1	Marked	Undernourished	100.4	34.0	35.4
52	39	Fair	7 yr	None	None	222.0	73.3	33.1
53	32	Poor	1	None	None	116.0	38.6	33.3
54	31	Poor	20 yr	None	None	165.2	51.6	31.8
55	55	Good	1 yr	None	None	152.3	45.6	29.7
56	29	Good	2 yr	None	None	144.9	48.5	33.3
57	27	Excell	4 days	Marked	None	136.4	41.4	30.3
58	34	Fair	13 yr	None	None	176.0	42.4	23.9
59	32	Good	1	Moderate	None	142.2	46.6	32.8

## SUMMARY

A study of free and total cholesterol values for the blood serum of patients suffering with peptic ulcer has been presented. A total of 59 patients were used, of whom 45 were males and 14 females.

1 The range of total serum cholesterol values was from 98.1 mg per cent to 300 mg per cent, with a mean value of  $160.8 \pm 38.7$  mg per cent (S.D.).

2 The range of free cholesterol values was from 32.1 mg per cent to 83.7 mg per cent with a mean value of  $47.7 \pm 11.1$  mg per cent.

3 The range of percentage free cholesterol was from 24.4 per cent to 38.6 per cent with a mean value of  $30.4$  per cent  $\pm 4.1$  per cent.

4 These determinations were made by the Schoenheimer and Sperry technique with modifications as noted above, and were compared with normal values obtained by Sperry, with the following results. There is a significant lowering of the mean value for total cholesterol, with a rise in the percentage of free indicating the fall in total cholesterol to be due mainly to a fall in esters.

5 Since almost all of these patients were on a high fat diet, the lowered blood cholesterol values tend to favor the theory that these levels are independent of exogenous food metabolism.

We wish to express our indebtedness to Dr. W. M. Sperry of the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, for his untiring interest and aid in this study.

## REFERENCES

- 1 Schoenheimer, R., and Sperry, W. M. A Micromethod for the Determination of Free and Combined Cholesterol, *J Biol Chem* 106 745, 1934.
- 2 Fitz, F. The Application of the Colorimeter to the Schoenheimer Sperry Method for the Determination of Free and Combined Cholesterol, *J Biol Chem* 109 23, 1935.
- 3 Shapiro, A., Lerner, H., and Posen, E. A Fixed Color Standard for Cholesterol Determinations, *Proc Soc Exper Biol & Med* 32 1300, 1935.
- 4 Sperry, W. M. The Relationship Between Total and Free Cholesterol in Human Blood Serum, *J Biol Chem* 117 341, 1937.
- 5 Cantarow, A. Progress in Cholesterol Metabolism, *Internat Clin* 2 237, 1935.
- 6 Stone, A. Clinical Importance of Cholesterol, *Southern M J* 28 706, 1935.
- 7 Feraru, F., and Offenkrantz, F. M. A Study of Cholesterol Values in Luetics, *Am J Syph Gonorr & Ven Dis* 21 267, 1937.
- 8 Stoesser, B. Cholesterol Studies in Acute Infections, *Proc Soc Exper Biol & Med* 32 1324, 1935.
- 9 Offenkrantz, F. M., and Karshan, M. A Study of Serum Cholesterol Values in Normal Children, *Am J Dis Child* To be published.
- 10 Blotner, A. Blood Fat Tolerance Tests in Malnutrition and Obesity, *Arch Int Med* 55 121, 1935.
- 11 Boyd, E. M. Diurnal Variations in Plasma Lipids, *J Biol Chem* 110 61, 1935.
- 12 Okey, R., and Stewart, D. Diet and Blood Cholesterol in Normal Women, *J Biol Chem* 99 717, 1927.

## A STUDY OF SERUM CHOLESTEROL IN PATIENTS WITH PEPTIC ULCER\*

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IN REVIEWING the literature, we have been unable to find any record of free and total cholesterol determinations in the blood serum of patients with peptic ulcer. We are therefore reporting a series of such determinations, with the hope that the results may be of some significance in further understanding the pathogenesis of this condition.

The study was carried out on patients either admitted to the medical and surgical wards of the Presbyterian Hospital, or under observation in the Vanderbilt Clinic. No case is included in which confirmation of the clinical diagnosis of peptic ulcer was not obtained by x-ray study after a barium meal (deformity of the mucosa, or visible crater).

This series included a total of 59 patients, of whom 7 were on the surgical services, 9 ambulatory to the clinic, and 33 on the medical services. The total age range of these patients, of whom 45 were male and 14 female, was from twenty-two to seventy-one years, but 46 patients fell between thirty and fifty years of age. Seven of these patients had a gastric ulcer, while 52 had a duodenal ulcer.

Samples of blood were obtained by venipuncture from the patients who had been fasting for a minimum of ten hours. In every instance the blood was obtained as soon as possible after the patient was admitted to the wards or clinic, in order to reduce as far as possible the length of time that the patients were on a high fat diet. As part of our procedure for all groups of determinations, the specimens were collected in 15 c.c. tubes and placed in the refrigerator within an hour, the tubes being tightly stoppered. Twenty-four hours later the clot was rimmed with a thin glass rod, and the samples centrifuged for fifteen minutes at 1,500 r.p.m. The serum was pipetted into tubes and recentrifuged for five minutes to insure removal of cells. The determination of free and total cholesterol in the serum was carried out by the method of Schoenheimer and Sperry,<sup>1</sup> with the use of the colorimeter as described by Fitz<sup>2</sup> and the ink standard originated by Shapiro and others.<sup>3</sup>

The statistical procedures employed are as follows:

1. Standard Deviation of a Single Observation ( $\sigma$ ) =  $\sqrt{\frac{\sum d^2}{N}}$ .

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For all determinations except differential counts, a 4.5 cc sample of blood was mixed with 0.5 cc of an anticoagulant mixture containing 12 per cent ammonium oxalate and 0.8 per cent potassium oxalate. This mixture gives a resulting percentage of the two salts which does not change the size of the red cells. Sedimentation on whole blood and suspensions of 5 million, 4 million, 3 million and 2 million cells in plasma was determined in Wintrobe tubes, filled to the 50 mm mark. Readings were made at the end of one hour, two hours, and three hours. Occasionally, it was difficult to obtain an accurate reading in the tube containing the two million suspension at the end of one and two hours, but usually the level was clear cut at the end of three hours. Therefore, only the three hour reading is here reported. The suspensions were made as follows. If the red cell count was over 5 million cells per c mm of plasma, 2.0 cc of the blood anticoagulant mixture was retained, the remainder centrifuged. If the red cell count was less than 5 million cells per c mm, one hematocrit tube was filled to the 50 mm mark and the remainder centrifuged at low speed. Sufficient of the supernatant fluid was added to or removed from the retained specimen to produce a resulting suspension of 4.5 million cells per c mm of the plasma anticoagulant mixture. This is equivalent to a suspension of 5 million cells per c mm of plasma alone. Four-tenths cubic centimeter of this suspension was mixed with 0.1 cc of supernatant fluid to produce a suspension equivalent to a four million suspension of cells in plasma. Subsequent dilutions in similar manner produced suspensions equivalent to three and two million cells per c mm of plasma. This method of making suspensions is that used by Walton.<sup>6</sup> Red and white cell counts were made on the original blood anticoagulant mixture in certified pipettes and counting chambers, correction being made for the 9:1 dilution. Hemoglobin was determined on the same specimen in the Haden Hauser's hemoglobinometer, Clinical model, 1935, the same correction being made for dilution. The Wintrobe tube, filled to the 50 mm mark with blood anticoagulant mixture, and previously used for the determination of sedimentation rate of whole blood, was centrifuged at 3000 r p m for twenty minutes for the determination of red cell volume, using the same correction as in the other determinations.

Differential counts were made on direct smears stained with a modification of the Jenner stain devised by William A. Groat.<sup>14</sup> In each instance 200 to 300 cells were counted, enumerating the percentage of filamented amphophiles, non filamented amphophiles, lymphocytes, large mononuclears, eosinophiles, and basophiles. The criterion for differentiating filamented and nonfilamented cells is that used by Cook and Ponder and revised by Farley, St. Clair, and Reisinger.<sup>9</sup>

Total blood proteins were determined on three experiments. For this determination, 5.0 per cent sodium oxalate was used as anticoagulant, the protein estimated according to the colorimetric method described by Hawk and Bergem.<sup>10</sup>

Three animals were autopsied after varying periods of anemia, three allowed to recover.

*Results*—In Table I are shown the complete data on a typical animal subjected to repeated bleedings and allowed to recover (Expt. 36.5). Fig. 1 shows

### Free and Total Cholesterol Values for Peptic Ulcer Series; Frequency Curve

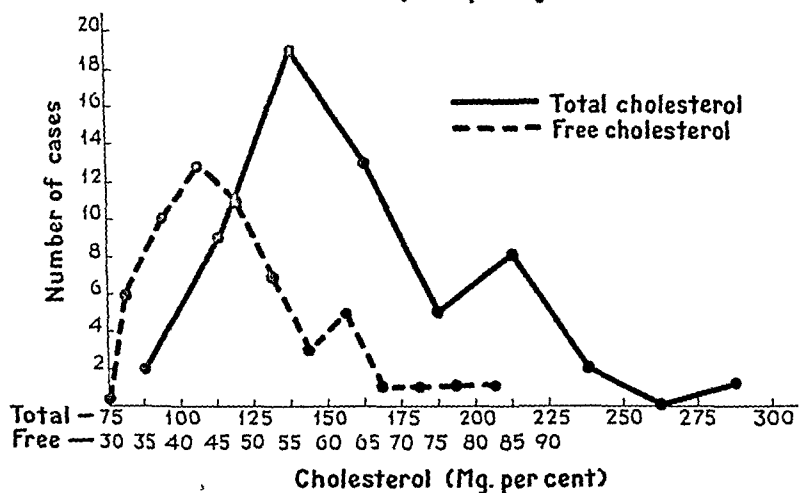


Fig. 1.

### Total Serum Cholesterol Values for Normal Adults (Sperry's Series)

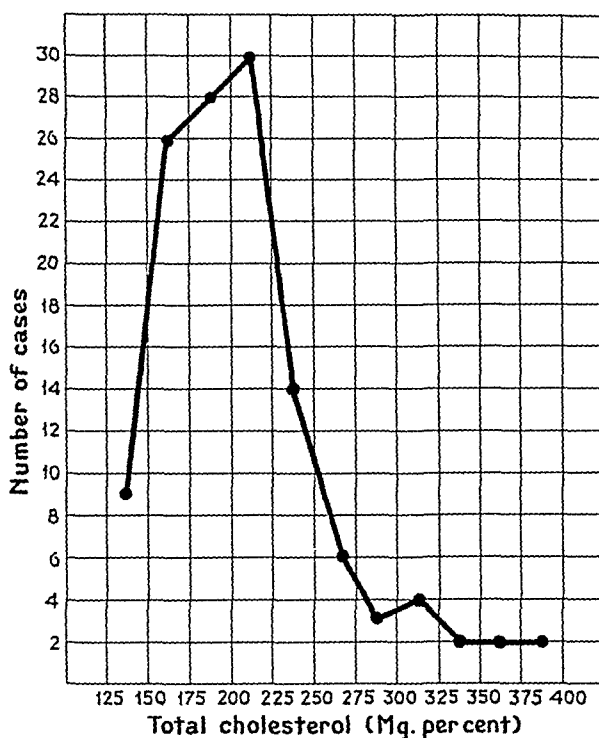


Fig. 2.

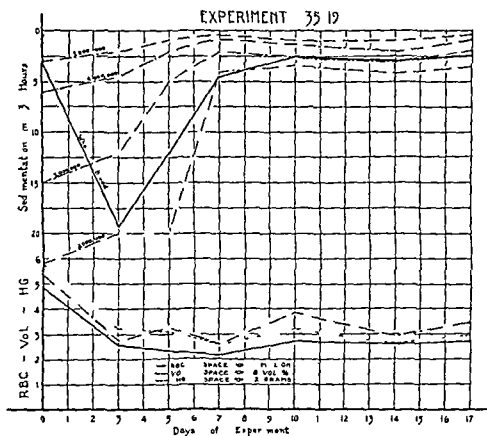


Fig. 1.—The top graph shows the sedimentation of whole blood and suspensions of 5 million 4 million 3 million and 2 million cells in plasma. The lower lines show the red blood count volume per cent and hemoglobin. Note the gradual slowing of the sedimentation of the suspensions.

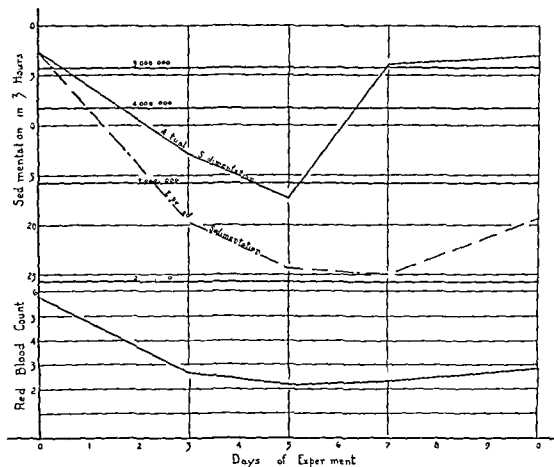


Fig. 3.—The top black line represents the average sedimentation of whole blood of the four experiments (Expers 35 19 36 3 36 4 and 36 5). The shaded line shows the expected sedimentation at that blood count on the basis of the readings on suspensions at the beginning of the experiments. The average values of sedimentation of the various suspensions at the beginning of the experiments is shown by the transverse lines labeled 5 000 000 4 000 000 3 000 000 and 2 000 000. The lower graph records the red cell counts in millions throughout the first ten days of these experiments.

TABLE II  
ANALYSIS OF CASES

NO.	AGE	NUTRI- TION	DURATION SYMPTOMS	ANEMIA	ADDITIONAL REMARKS	CHOLESTEROL		
						TOTAL MG. %	FREE MG. %	% FREE
1	45	Poor	7 mo.	None	Osteoarthritis Gastroenterostomy	102.3	34.5	33.4
2	22	Good	6 yr.	Moderate	None	124.0	48.0	38.6
3	29	Fair	5 yr.	None	None	149.0	46.6	31.3
4	45	Poor	7 yr.	Severe	Perf. and gastro- enterostomy	144.8	36.8	25.1
5	71	Poor	1 yr.	Moderate	None	226.0	83.7	35.5
6	47	Excell.	12 yr.	Moderate	None	195.9	51.9	26.5
7	40	Fair	8 yr.	None	Lost 10 pounds, 6 mo.	220.0	53.3	24.2
8	50	Fair	6 yr.	None	Pyloric constrict.	200.0	64.6	32.3
9	46	Good	10 yr.	None	None	204.8	65.3	30.8
10	40	Fair	6 yr.	None	Hypertensive cardiac disease	156.8	42.1	26.8
11	36	Good	7 yr.	Severe	Transfused 4 days prior to	154.8	42.5	27.4
12	47	Excell.	24 yr.	None	None	139.7	46.6	39.1
13	37	Fair	12 yr.	None	None	133.6	48.2	35.6
14	44	Good	5 yr.	None	None	185.5	60.2	32.4
15	49	Poor	12 yr.	None	None	201.2	60.1	30.0
16	26	Good	1 mo.	None	None	145.2	46.6	32.3
17	63	Fair	10 yr.	Slight	None	98.1	32.1	32.6
18	49	Fair	5 yr.	None	Syphilis, cured 1925?	127.5	36.8	28.0
19	31	Good	5 yr.	None	Gastroenterostomy 2/36	145.2	44.2	30.4
20	36	Poor	?	None	None	149.0	42.2	28.2
21	34	Poor	2 yr.	Moderate	None	170.6	55.7	32.6
22	31	Poor	9 yr.	None	Spastic colon	151.0	40.4	26.5
23	59	Poor	23 yr.	Marked	Marked cachexia	171.6	45.0	27.0
24	39	Poor	1 mo.	None	None	218.2	64.0	29.4
25	42	Poor	7 yr.	None	None	143.4	37.5	25.7
26	47	Good	3 wk.	Marked	None	130.4	40.1	30.7
27	52	Good	2 yr.	Moderate	None	173.9	53.8	30.9
28	41	Fair	10 yr.	None	None	146.2	39.1	26.5
29	24	Poor	2 yr.	Marked	Hematuria (cause unknown)	121.6	37.1	30.5
30	26	Good	5 days	Moderate	Migraine	147.3	43.4	29.4
31	42	Fair	?	None	None	117.8	38.2	32.4
32	31	Fair	8 yr.	Marked	Syphilis, cured	109.2	34.0	31.3
33	33	Poor	6 wk.	None	None	241.6	61.5	25.3
34	35	Good	6 yr.	None	None	159.6	36.9	23.1
35	50	Poor	11 yr.	Marked	Carcinoma of stomach also	208.0	51.4	24.6
36	39	Good	12 yr.	None	Syphilis, 1924, cured	150.1	41.6	27.7
37	55	Poor	2 yr.	Marked	None	300.0	79.8	26.6
38	39	Good	11 yr.	None	None	139.8	44.3	31.6
39	47	Poor	10 yr.	None	None	147.9	44.8	29.9
40	54	Poor	32 yr.	Marked	Died of pneumonia 4 days after	120.4	39.0	32.6
41	42	Good	2 yr.	None	None	120.1	33.6	28.0
42	56	Poor	26 yr.	None	None	214.2	58.2	28.0
43	25	Good	5 yr.	Marked	None	201.2	52.6	26.2
44	29	Poor	4 yr.	None	None	121.4	34.5	31.3
45	32	Fair	3 mo.	Marked	Cryptorchid (uni- lateral)	166.0	49.9	30.1
46	60	Poor	2 yr.	Moderate	None	138.2	47.4	34.3
47	66	Fair	?	None	Healed T. B.	162.7	53.8	32.6
48	36	Fair	9 yr.	None	None	190.0	58.1	30.5
49	61	Fair	1 yr.	Moderate	Hyper. Cardiac dis. Hypertrophy prostate	140.0	38.3	27.9
50	45	Poor	2 yr.	None	None	168.0	48.0	28.6

TABLE II  
EXPERIMENT 3519  
Effect of anemia due to hemorrhage upon the sedimentation rate  
Experiment begun on Feb 12, ended March 26  
Male, white rabbit, weight 3,400 grams

DAY	BLED CC	VOL %	HGB GR PER 100 CC	RBC MILLIONS	SEDIMENTATION IN MM (3 HR)					WBC THOU SANDS	FIL %	NON FIL %	LYMPH %	MONO %	EOS %	BAS %	PLASMA PROTEIN GR PER 100 CC	WT
					WHOLE BLOOD	SUSPENSIONS												
						5	4	3	2									

0	650	43.0	11.8	4.93	3.0	3.0	6.0	15.0	23.0	10.2	21.3	12.0	62.7	1.0	0.7	2.3	6.0	3400
1	550																	
3	550	22.0	6.4	2.60	19.0	2.0	4.5	12.0	20.0	10.3	20.3	7.3	64.4	1.0	0.7	5.3		3350
5	450	25.5	6.2	2.25	12.0	1.0	2.0	5.0	20.0	8.9	8.7	4.3	77.3	0.3	0.3	9.4		
7	430	21.0	5.2	2.10	4.0	0.5	1.0	2.0	3.5	5.5	17.7	8.0	67.0	1.3	0.7	5.3		
10	550	31.0	6.5	2.73	25*	1.0	1.5	2.5	3.0	4.6	10.0	5.3	78.3	0.0	1.0	5.5	7.1	3500
13	550																	
14	100	23.5	5.2	2.60	3.0	1.0	2.0	3.0	4.0	6.2	8.3	4.7	82.3	0.0	1.0	5.7	5.8	
15	400																	
17	540	27.0	5.9	2.70	25*	0.5	1.0	2.0	3.5	7.2	22.5	10.5	57.0	0.5	2.5	7.0	5.6	
19	260																	
21	430	24.0	5.8	3.32	3.0	1.0	1.5	3.0	3.0	3.3	17.3	4.0	72.0	0.3	2.7	2.0	6.2	
24	450																	
28	600	34.0	7.5	4.60	1.0	1.0	1.5	2.0	2.0	8.0	15.0	4.5	59.5	1.0	2.0	18.0	5.7	
31	450																	
33	350																	
35	600																	
38	100	29.0	6.2	4.20	2.0	1.5	2.0	3.0	3.5	5.9	14.0	3.0	79.0	2.0	1.0	1.0	4.9	
41	470																	
43	150	28.0	5.7	4.46	2.0	2.0	2.5	4.0	5.0	7.3	22.5	7.5	62.0	1.0	1.5	6.5		3525

\*Sedimentation calculated from suspensions not determined on whole blood

## THE SEDIMENTATION RATE IN EXPERIMENTAL ANEMIA (RABBIT)\*

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THE usefulness of the sedimentation test rests on the assumption that the speed of sedimentation is in direct proportion to the degree of tissue damage. Obviously the conditions under which the test is performed must be controlled, or an erroneous interpretation may be given the result of the procedure.

The effect of anemia alone upon the rate is not altogether clear. All workers are agreed that severe grades of anemia in the human subject are accompanied by accelerated rates. In less severe grades of anemia there is still some discussion as to whether the speed of sedimentation increases in direct proportion to the degree of anemia or not. Fåhræus<sup>1</sup> and Cutler<sup>2</sup> have expressed the view that in the absence of other pathology there is some compensatory mechanism present which tends to keep the sedimentation rate relatively constant in moderate anemia. Gram,<sup>3</sup> Rourke and Ernstene,<sup>4</sup> Wintrobe,<sup>5</sup> and Walton,<sup>6</sup> on the basis of dilution experiments with cells in their own plasma, have been able to construct graphs showing the relation of various grades of anemia to sedimentation rate. It is important to realize that they were using suspensions of normal cells in normal plasma, not true anemic blood. We are presenting the results of experiments showing the behavior of the sedimentation rate in the anemia of acute and chronic hemorrhage and in recovery from that anemia.

It will be shown by successive readings on the *sedimentation of whole blood* that there is a marked increase in sedimentation rate in acute anemia, but only a slight increase in chronic anemia. Results of observations on the *sedimentation of suspensions of cells in plasma* will show the quantitative change in the factors affecting the sedimentation rate which are attendant upon the anemia produced. In general there will be noted a slowing of the sedimentation of suspensions as the anemia progresses and an acceleration in recovery.

*Methods.*—Six male rabbits were used, four New Zealand whites, weighing from 2,350 to 3,500 gm., and two Champagnes d'Argents, weighing 3,300 and 3,500 gm. The animals were housed in individual cages and fed a diet of oats and celery throughout the course of the experiments. On the first and second days of each experiment, the animal was bled from the ear vein approximately 15 to 20 c.c. of blood per kilo of body weight. Subsequently, he was bled approximately three times a week to maintain a fairly constant degree of anemia. Sedimentation rate, hemoglobin, hematocrit value, complete red and white cell counts, and differential counts were determined on the first and second days, usually three times a week for the first two weeks, twice the third week, and approximately once a week thereafter.

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gradual slowing of the rate in both the whole blood and in the various suspensions. On the seventh day in four experiments the rate of sedimentation of the anemic blood, as drawn, closely approximated that found in normal whole blood at the beginning of the experiments. The omission of the reading between the sixth and eleventh days in Exper 361 probably meant the missing of the first slowing of the rate. One ear was accidentally burned in Exper 362, and this was reflected in a change in the rate.

In recovery from the anemia (Expers 361, 364, and 365), there was a gradual acceleration of sedimentation of the various suspensions. The rates found in whole blood remained slow. The rate of suspensions in Exper 361

TABLE V  
EXPERIMENT 362  
Male White Rabbit

DAY	BLED CC	VOL %	HGB GR I LR 100 CC	RBC MILLIONS	SEDIMENTATION					WEIGHT
					WHOLE BLOOD	SUSPENSIONS MILLIONS				
						5	4	3	2	
0	46	36.0	9.8	4.80	4.0	4.0	9.0	13.0	22.0	2560
1	41									
*3	39	24.0	5.4	2.70	14.0	2.0	4.0	8.0	-	
X5	40	24.5	5.8	2.90	3.0+	0.5	2.0	2.5	3.5	
H8	44	25.5	5.6	2.76	6.0	0.5	5.0	8.0	13.0	
12	38	28.8	5.8	3.10	3.5	0.5	1.5	3.0	6.0	
15	50	27.0	5.9	3.30		1.5	2.0	3.0	4.0	2300
19	31	29.0	7.2	4.24	2.0	1.0	2.0	3.5	4.0	
22	30	29.0	6.9	3.70	2.5	2.0	2.5	3.0	3.5	
26	23									
27	30									
29	40	24.5	6.4	3.10	3.0+	1.0	2.0	3.5	4.5	
33	25	-	4.8							
36	30	31.0	6.2	3.66		1.0	1.0	2.0	3.0	
41	35	27.0	6.1	4.20	2.5	2.0	2.5	3.5	4.5	
43	22									
46	28	-	5.4							2550
48	10	18.0	4.4	3.10	2.0	2.0	2.5	3.0	3.5	
Autopsy										

\*Marked redness about base of left ear

X Demarcating crusted area approximately 1.0 cm in diameter over the left ear

H Black slough 1.0 cm in diameter at base of ear

TABLE VI  
EXPERIMENT 363  
Male Gray Rabbit

DAY	BLED C C	VOL %	HG GR PER 100 C C	R B C MILLIONS	SEDIMENTATION					PLASMA PROTEIN	WT
					WHOLE BLOOD	SUSPENSIONS MILLIONS					
						5	4	3	2		
0	65	49.0	13.8	5.80		30	50	110	230	76	3550
1	70				25						
3	62	20.5	5.3	2.19	120	20	30	50	120	71	
5	36	18.0	4.2	1.90	360	15	20	50	220	59	
7	10	16.0	4.4	1.90	40	10	20	40	60	72	
10	15	19.0	3.9	2.26	30+	-	20	40	50		
16	10	24.5	5.3	4.10	20	15	20	25	30		
21	10	30.0	5.8	4.25	20	15	20	30	30	49	
24	10										
27	10	24.0	5.4	4.36	-	25	25	30	40	50	2950

in graphic form the relation of red cell count, volume, and hemoglobin to the sedimentation rate of whole blood and suspensions of cells in plasma in this animal.

In Table II are shown the complete data on a typical animal subjected to repeated bleedings and autopsied on the forty-third day. Fig. 2 is a graphic representation of the relation of the red cell count, volume, and hemoglobin to the sedimentation of whole blood and of suspensions of the cells in plasma for the first seventeen days of the experiment.

Tables IV to VII show the abbreviated data on the remaining four experiments with omission only of the white blood counts and differential counts.

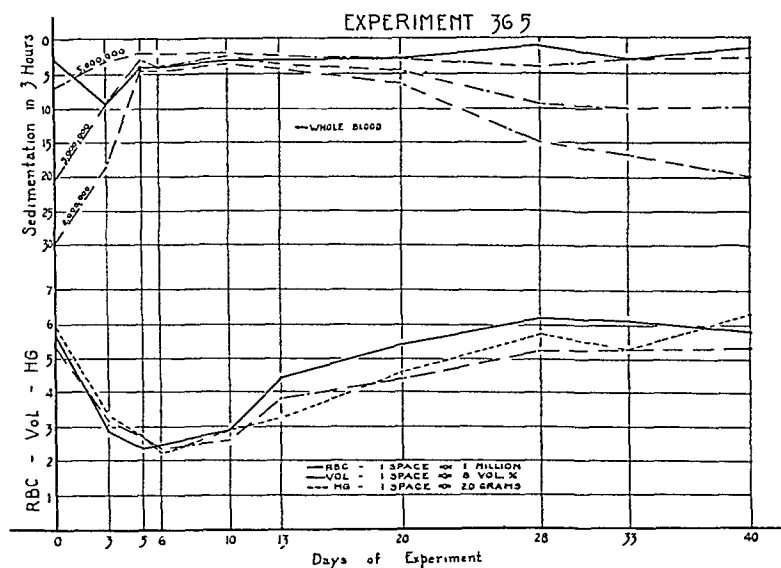


Fig. 1.—The top graph shows the sedimentation of whole blood and of suspensions of 5 million, 3 million, and 2 million cells in plasma. The lower graph shows the red cell count volume per cent and hemoglobin at the various stages in the experiment.

Table III shows the relation of red cell count and sedimentation rate in all six animals for the first eleven days. Fig. 3 shows the relation of the average actual sedimentation observed in four animals (Expers. 35.19, 36.3, 36.4, and 36.5) with the expected rate on the basis of dilution of cells in plasma at the beginning of the experiments. In construction of the graph, Exper. 36.1 was disregarded because there were no readings between the sixth and eleventh days. Experiment 36.2 was omitted, since one ear was accidentally burned on the fifth day, and this was reflected in an increased rate. Throughout the remainder of their course these two animals gave sedimentation rates which closely paralleled the values observed in the other four animals.

In all six experiments there was noted an acceleration of the sedimentation rate of whole blood on the third day following the initial bleeding. This increase approximated the rate found in a suspension of cells of similar concentration at the beginning of the experiment. In the following readings there was seen a

Microscopic examination showed marked diffuse hyperplasia of all marrow sections (femora). The erythroblastic series appeared to predominate.

Although the number of animals used in this work is small, the uniformity of results appears to justify this report.

#### SUMMARY

The following changes in the sedimentation rate were observed in anemia produced by hemorrhage in rabbits:

1 In *acute anemia* an *acceleration* of the sedimentation rate of *whole blood* in nearly direct proportion to the degree of anemia,

2 In *chronic anemia* a gradual *slowing* of the sedimentation rate of both *whole (anemic) blood* and of *suspensions* of cells in plasma, which reached a relatively constant slow rate on and after the seventh day,

3 In *recovery* from chronic anemia a gradual *acceleration* in the sedimentation rate of *suspensions* of cells in plasma, which lagged behind the rise in red cell count.

Changes in the red cells were similar to those noted by other observers.

No significant changes were found in the total white blood count, differential count or in the filament/nonfilament ratio.

#### REFERENCES

- 1 Fåhræus, Robin. The Suspension Stability of the Blood, *Physiol Rev* 9: 241, 1929.
- 2 Cutler, J. Personal communication, 1935.
- 3 Gram, H. C. Sedimentation of Blood Corpuscles in Various Internal Diseases and Result of Correction of This Value for Variations of Hemoglobin Percentage, *Acta med Scandinav* 70: 242, 1929. Abstr. in *J. A. M. A.* 92: 2070, 1929.
- 4 Rourke, M. D., and Ernestine, A. C. A Method for Correcting the Erythrocyte Sedimentation Rate for Variations in the Cell Volume Percentage of Blood, *J. Clin. Invest.* 8: 545, 1930.
- 5 Wintrobe, M. M., and Lindsberg, W. A Standardized Technique for the Blood Sedimentation Test, *Am. J. M. Sc.* 189: 102, 1935.
- 6 Walton, A. C. R. The Corrected Erythrocyte Sedimentation Test, *J. Lab. & Clin. Med.* 18: 711, 1933.
- 7 Heller, V. G., and Paul, H. Changes in Cell Volume Produced by Varying Concentrations of Different Anticoagulants, *J. Lab. & Clin. Med.* 19: 777, 1934.
- 8 Haden Russell. New Clinical Model of the Haden-Hausser Hemoglobinometer, *J. Lab. & Clin. Med.* 20: 762, 1935.
- 9 Farley, D. R., St. Clair, H., and Reisinger, J. H. The Normal Filament and Non-filament Polymorphonuclear Neutrophil Count, Its Practical Value as a Diagnostic Aid, *Am. J. M. Sc.* 180: 336, 1930.
- 10 Hawk, Philip B., and Bergheim, Olaf. *Practical Physiological Chemistry*, ed. 10, Philadelphia, P. Blakiston's Son and Co., Inc., pp. 449, 450.
- 11 Price Jones, Cecil. Observations on the Changes Produced in the Blood and Bone Marrow by Hemorrhage and Phenylhydrazine. Part 1. *J. Path. & Bact.* 15: 4, 1911.
- 12 Whipple, G. H., Hooper, C. W., and Robscheit, F. S. Blood Regeneration Following Simple Anemia. I. Mixed Diet Ration. *Am. J. Physiol.* 53: 151, 1920.
- 13 Osgood, E. E., and Haskins, H. D. Causes, Classification and Differential Diagnosis of Anemias Based on the Detailed Examination of Over Two Hundred Patients and a Study of the Literature, *Ann. Int. Med.* 5: 1367, 1932.
- 14 Groat, W. A. A General Purpose Polychrome Blood Stain, *J. Lab. & Clin. Med.* 21: 978, 1936.

TABLE I  
EXPERIMENT 305  
Effect of anemia due to hemorrhage upon sedimentation rate.  
Experiment begun on March 14 and ended April 23.  
Male, white rabbit, weight 2,350 grams.

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	W.B.C. THOU- SANDS	SEDIMENTATION IN MM.										FIL. %	NON- FIL. %	LYMPH. %	MONO. %	EOS. %	BAS. %	PLASMA PROTEIN GR. PER 100 C.C.	WT.
						WHOLE BLOOD					SUSPENSIONS												
						5	4	3	2	1	5	4	3	2	1								
0*	40.0	43.0	12.0	5,72	6,2	3.0	7.0	13.0	21.0	30.0		23.0	8.0	63.5	1.5	1.5	2.5		2350				
1	25.0																						
3	10.0	25.0	6.4	2,93	5,3	9.0	3.0	5.0	9.0	18.0		19.3	2.3	72.7	1.3	1.3	3.0	4.9					
5	20.0	22.0	5.4	2,34	4.5	4.0	2.0	2.0	3.0	4.5		15.0	5.0	73.5	1.0	1.5	4.0						
6	15.0	19.0	4.4	2,44	5.5	4.0	2.0	3.0	4.0	4.5		13.0	3.0	76.5	1.5	1.0	5.0	4.7					
10	25.0	22.0	5.8	2,94	4.7	3.0	2.0	2.0	2.5	3.5		9.5	1.5	83.5	0.0	3.0	2.5	5.5					
13*	10.0	31.0	6.5	4,46	5.1	3.0†	2.5	3.0	3.5	4.0		9.0	2.0	81.5	2.0	1.5	4.0	6.1					
20	4.5	35.0	9.0	5,40	6,1	3.0	3.0	4.5	4.5	6.5		9.0	4.5	77.0	3.0	0.0	6.5		2075				
28	4.5	42.0	11.4	6,18	5.7	1.5	4.0	5.5	9.0	15.0		13.5	5.0	70.5	5.5	1.0	5.5						
33	4.5	42.0	10.5	6,15	8.0	3.0	3.0	5.0	10.0	17.0		22.5	7.5	41.0		1.0	22.5		2100				
40	4.5	44.0	12.8	5,87	6,2	2.0	3.0	6.0	10.0	20.0													

\*Photographed

†Sedimentation calculated from results of suspensions, not determined on whole blood.

## FEVER THERAPY METHODS

The first report of fever therapy being used in the treatment of mental disorders in the United States appeared in 1922 at Saint Elizabeth's Hospital, Washington, D C Since that time, a large number of mental institutions in this country have inaugurated fever therapy as a treatment for various psychoses

A number of theses have been written on the various methods of producing artificial fever with malaria, ratbite fever organisms, typhoid vaccine, Coley's solution, sulphosin, diathermy, radiotherapy, radiant energy, and hydrotherapy All these methods have reported various degrees of success Each method seems to bring out a new theory as to the reaction that causes the fever and the effect it has on the patient

To observe some of these factors produced by artificial fevers, we have used for the past two years typhoid paratyphoid vaccine prepared by Parke, Davis Company The vaccine contains 2,000 million killed bacteria per cubic centimeter prepared specially for intravenous injections The vaccine is easily diluted, readily administered, and has no drastic effects The injections are given intravenously once a week The initial dose in most cases is from 10 to 20 million bacteria, depending upon the weight of the patient The stock vaccine is diluted with sterile triple distilled water and the desired amount given with a tuberculin syringe

We have found that the most desirable results are obtained by adhering to the following schedule of injections

- 10 million bacteria the first week
- 20 million bacteria the second week
- 40 million bacteria the third week
- 80 million bacteria the fourth week
- 100 million bacteria the fifth week
- Increasing the dose a hundred million bacteria each week until eighteen doses have been given

This schedule should be applied only in psychotic cases where there is no evidence of an active somatic disease Cecil (1935) gives a complete list of important contraindications which should be considered previous to the intravenous use of protein therapy

## THE PRESENT STUDY

During the past two years one hundred selected patients with various psychoses have received typhoid paratyphoid vaccine therapy What is meant by selected patients is that only those individuals who were free from any somatic diseases received the therapy In our group there were 37 males and 63 females The average age was thirty six years The oldest patient was sixty eight and the youngest was sixteen The average time that the entire group had been in their psychosis when the therapy started was five years and six months

The literature on the regulation of the blood elements by the vegetative nervous system was excellently summarized by Ferdinand Hoff in 1928

TABLE III

THE RELATION OF THE RED BLOOD CELL COUNT TO SEDIMENTATION OF WHOLE BLOOD.  
ALL SIX EXPERIMENTS

DAY	EXPER. 35.19		EXPER. 36.1		EXPER. 36.2		EXPER. 36.3		EXPER. 36.4		EXPER. 36.5	
	R.B.C. MIL- LIONS	SED. MM.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.	R.B.C.	SED.
0	4.93	3.0	5.34	3.0	4.80	4	5.80	2.5	6.50	2.0	5.72	3.5
3	2.60	19.0	3.36	8.0	2.70	14†	2.19	12.0	2.60	12.0	2.93	9.0
5	2.25	12.0			2.90	3†	1.90	36.0			2.34	4.0
6			3.50	3.5*							2.44	4.0
7	2.10	4.0					1.90	4.0	2.25	4.0		
8					2.76	6†						
9									3.40	3.0		
10	2.73	2.5					2.26	3.5			2.94	3.0
11			4.37	1.5					2.30	3.0		
12					3.10	3.5						
13			3.12	3.5								

\*Readings estimated from results of suspensions, not actually determined on whole blood.

†Ear showed some burn.

In construction of the graph in Fig. 3 only values actually found on the 0, 3, 5, 7, and 10th days were used (Expers. 35.19, 36.3, 36.4, and 36.5).

R. B. C. recorded in millions.

Sedimentation recorded in millimeters.

TABLE IV

EXPERIMENT 36.1  
Male White Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					WEIGHT
					WHOLE BLOOD	SUSPENSIONS MILLIONS				
						5	4	3	2	
0	30	39.0	11.6	5.34	-	3.0	5.0	8.0	12.0	2510
1	28									
3	5.0	25.0	7.0	3.36	-	3.0	5.0	10.0	1.0	
6	27.0	31.0	8.0	3.50	-	2.0	3.0	4.0	13.0	
11	39.0	36.0	9.0	4.37	1.5	1.5	2.5	3.0	5.0	
13	22	29.0	7.2	3.12	3.5	0.5	2.0	3.5	5.0	
17	54	34.0	9.0	4.09	2.5	1.5	2.5	3.0	4.0	
20	48	29.0	7.5	2.99	3.0	1.0	2.0	3.0	5.0	
34	30	28.0	6.8	3.30	-	1.5	2.0	3.0	4.0	
37	35	29.0	6.9	3.50	-	1.0	2.0	3.0	4.0	2460
41	30	33.0	8.0	3.42	2.0	1.5	2.0	3.0	5.5	
44	38	36.0	7.0	3.40	7.0	1.0	2.0	2.5	4.0	
46	28									
48	33	-	7.5							
51	15	26.5	7.0	3.50	2.0	2.0	2.5	4.0	3.0	
53	40									
55	38	29.0	7.5	3.84	2.5	1.5	2.0	3.0	4.5	
57	40									
59	29	-	5.4							
62	26	29.0	6.4	3.64	2.0	1.0	1.5	2.0	3.0	2625
64	33									
71	10	32.0	7.4	5.10	2.0	2.0	2.5	3.0	3.0	
76	10	42.0	10.0	5.09	1.0	1.0	1.5	2.5	2.5	
83	5	42.0	10.0	4.96	1.0	1.0	2.0	3.5	3.5	
90	5	48.0	12.0	6.64	1.5	1.5	2.5	3.5	5.0	
97	5	46.0	12.2	6.85	1.5	2.0	3.0	5.0	7.0	
104	10	49.0	13.8	7.92	1.5	3.5	4.5	10.0	12.0	
111	5	46.0	11.5	5.72	2.0	5.0	7.5	10.0	14.0	
119	5	41.0	11.6	8.92	2.5	-	6.0	10.0	15.0	

In a series of 148 cases of mental diseases of various kinds with different types of emotional states studied by Bowman and Kasanin (1929), the majority showed normal blood sugar values (80 to 120 mg dextrose per 100 cc of whole blood) in contrast to results of former investigators. The distribution curve was normal.

Blanche Labin (1927) reported on glycemia in the insane and stated that the variations from the normal limits were only slight.

Craig (1927) reported that no characteristic blood sugar curve was found in 90 patients with anxiety neuroses, epilepsy, and melancholia.

In 100 cases of manic depressive psychosis studied by Rothschild and Malamud (1931) and uncomplicated by somatic diseases, there was no constant relation between the ratios of distribution of sugar, bromine, calcium, and chlorides.

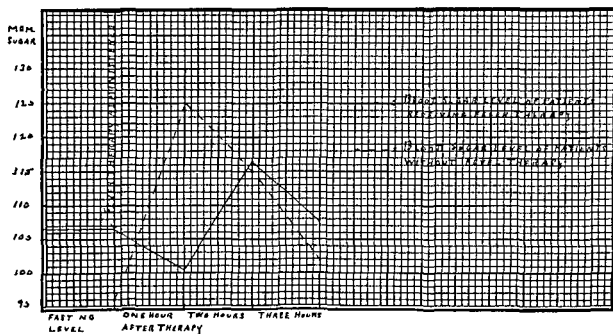


Chart 1

An extensive study presented by Piceman (1933) of the fasting blood sugar levels as disclosed by six samples taken at standard intervals from 59 male schizophrenic patients over a period of six and one half months, showed that 95 per cent of 347 determinations lay between the conventional limits of normality.

In dementia praecox patients treated with sulphur, Isikowitz (1934) points out the behavior of the blood sugar by showing that six hours after the injection of sulphosin there is a lowering of the sugar. The hyperthermic phase following the injection is frequently accompanied by hyperglycemia.

In our group of 100 cases of various psychoses, 30 patients were observed for changes in the blood sugar. For the determination of the blood sugar level at the various time intervals we used the approved method of John Wu. The first blood was taken before breakfast (fasting level), and immediately after the blood sample was removed, the patients received 6 ounces of whole milk, 2 soda crackers, and an intravenous injection of typhoid paratyphoid vaccine. Another blood sample was withdrawn at one, two, and three hours after the fever therapy.

at the end of the experiment was slightly faster than at the beginning. In the other two experiments, the rates did not quite increase to the original level. As noted by other workers,<sup>11, 12</sup> following acute blood loss there was a slight increase in both color and volume indices. In the period of chronic hemorrhage, the color indices fell more than the volume indices, giving saturation indices below 0.8, which has been shown to be characteristic of anemia of chronic blood loss.<sup>13</sup> In recovery the red cell count rose in each instance above the original level. In the animals kept anemic longer, this polycythemia was greater. The color indices rose gradually throughout the period of recovery to approximate the original values.

Tables IV, V, VI, and VII show the relation of red cell count, hemoglobin, and volume to sedimentation of whole blood and suspensions in the remaining experiments. The white blood picture showed no constant or significant change. The total count per cubic millimeter varied between 3,400 and 11,000, both extremes of which may be considered within the range of normal. Following the initial bleedings in two animals, there was a slight increase in the percentage of amphophiles, and in one animal an increase in nonfilamented amphophiles to over 50 per cent.

In three experiments plasma proteins were determined. These showed little or no change for the first seven days. Later there was an irregular decrease in the period of sustained blood loss.

Autopsy on three animals (Expers. 36.2, 36.3, and 35.19), after forty-eight days, twenty-seven days, and forty-three days, respectively, showed only generalized pallor of all the organs on gross examination. The marrow of the femora appeared very red and gelatinous. The tibial marrow was rather markedly reddened throughout the upper two-thirds, the remainder appearing rather fatty.

TABLE VII  
EXPERIMENT 36.4  
Male White Rabbit

DAY	BLED C.C.	VOL. %	HG GR. PER 100 C.C.	R.B.C. MILLIONS	SEDIMENTATION					PLASMA PROTEIN	WT.
					WHOLE BLOOD	SUSPENSIONS MILLIONS					
						5	4	3	2		
0	52.0	49.0	13.0	6,50	2.0	4.0	9.0	16.0	27.0	9.0	3700
1	33.0										
3	32.0	21.0	5.5	2,60	12.0	3.0	5.0	11.0	32.0	6.5	
5	25.0	21.0	5.5	3,30	-	-	-	-	-	8.0	
7	5.0	17.0	4.1	2,25	4.0	2.0	3.0	4.0	7.0	7.4	
9	10.0	16.0	3.9	3,40	4.0	3.0	3.0	3.5	5.0	4.9	
11	10.0	19.0	4.1	2,30	-	2.0	-	-	3.0	-	
13	10.0	22.0	3.75	2,67	3.0	1.0	2.0	2.5	3.0	4.8	
18	30.0	-	5.2	-	-	-	-	-	-	-	
20	10.0	20.5	3.9	3,40	2.5	1.5	2.0	2.5	3.0	6.1	
29	4.5	24.5	5.8	5,10	5.0	5.0	6.0	7.0	8.0	4.4	3425
36	4.5	27.0	6.5	5,70	5.0	4.0	6.0	8.0	13.0		
43	4.5	34.0	7.0	7,90	2.0	-	6.0	10.0	11.0		
50	4.5	35.0	8.0	6,63	2.0	4.0	8.0	12.0	18.0		
57	4.5	41.0	9.0	6,93	1.5	3.0	2.0	6.5	16.0		
71	9.0	39.5	12.0	7,90	1.0	2.5	3.5	7.0	6.0		
78	4.5	49.0	12.4	7,10	2.0	4.5	8.0	12.0	16.0		

carbonic acid from the blood in the expiration of abnormal amounts of carbon dioxide and creates the state designed as uncompensated deficit of carbon dioxide. This fact was demonstrated with hot baths by Haldane and Priestley (1905), later confirmed by Hill and Flack (1909) and a number of other workers. Hopkins (1934) reported the average fall of the carbon dioxide combining power to be 9.37 per cent by volume during hyperpyrexial baths.

The marked loss of carbon dioxide from the blood is of such a degree as to give rise to a condition of alkalosis which may be detected without difficulty in the rising pH values. This alkalosis during hyperpyrexia was reported by Cajori, Crouter and Pemberton (1923).

Barbour (1921) subjected dogs to hot baths and observed a dilution of the blood to be about 10 per cent. About this fact he was led to build his conception of water shifting between the tissues and the blood as a protection of the body against overheating. By this means larger quantities of blood were made available for filling the greatly dilated peripheral vascular bed, bringing more blood to the surface of the body for direct conduction and radiation of heat, as well as mobilizing water for loss of heat through evaporation of the sweat.

The period of well being which patients experience following typhoid paratyphoid therapy may well be accounted for by the stimulation of hemopoiesis and the shifting of water between the vascular system and the tissues.

#### THE EFFECTS OF THE DIVIDED DOSE METHOD OF FEVER THERAPY

Kirby (1926) reported that the beneficial effect of fever therapy in neurosyphilis was roughly proportional to the height of the temperature obtained. He believed that a temperature of about 105° F. was necessary in order to obtain the maximum results. Winslow Miller and Noble (1916) believed that a high temperature artificially produced, 104° F. or higher, has a favorable influence on an established infection while lower temperatures would seem to retard the formation of immune bodies. In order to produce artificially, temperatures of 104° F. or higher, Nelson (1931) used a technique which consisted in giving two daily intravenous injections of typhoid paratyphoid vaccine. He suggested that "the first dose be given at any selected time and be of a size calculated to cause slight fever. The second is given during the height of the fever produced by the first—usually at the end of the second or third hour. The second dose seems to have the effect of exploding the charge supplied by the first and in this way relatively small doses are capable of producing fever apparently as high as desired—105° F. to 107° F."

This method of daily divided doses of typhoid paratyphoid vaccine was used by Driver and Shaw (1933) with very satisfactory results in cases of neurosyphilis.

Eight of our patients received once a week a divided dose as suggested by Nelson. The highest temperature obtained by this method was 104.8° F. The average temperature of the group was 104.3° F.

The blood counts, differential and Arneth counts on the patients who were treated by the divided dose method showed that the maximum stimulation of the hemopoietic system is not in direct proportion to the height of the temperature. Therefore, the single dose method outlined above is recommended.

## SOME SYMPATHETIC FACTORS IN THERMOTHERAPY\*

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### INTRODUCTION

THE production of infectious diseases in mental patients was first attempted by Rosenblum in 1875. Patients who were suffering from different mental disorders were inoculated with relapsing fever organisms. Later, Von Jauregg studying the results of epidemics occurring in mental hospitals, noted that when these patients contracted infectious diseases accompanied by a high fever, the tendency in nearly every case was to recover from their psychosis.

Stimulated by this discovery, Von Jauregg, in 1887, first proposed the treatment of paresis by the malarial method. In the beginning he realized the severity of this method of treatment and resorted to various toxins to produce artificial fever. The results obtained from the use of various toxins were not entirely satisfactory and he returned to his original idea of malarial inoculations.

Torres in 1913 reported successful results in the treatment of typhoid fever by giving typhoid vaccine intravenously and described the sharp reactions that followed its injection. Typhoid vaccine had been used previous to this in the treatment of typhoid, but the injections had always been given subcutaneously.

Dessy, Grapiolo, and Fossati in 1914 published a paper on the treatment of typhoid fever with typhoid vaccine. The authors compared the results obtained by the subcutaneous and intravenous injections of typhoid vaccine. They concluded that the intravenous method gave much quicker results.

Kraus and Mazza in 1914 treated typhoid fever with intravenous injections of typhoid vaccine and noted that the mortality rate was definitely reduced. Ichikawa also reported favorably on this form of treatment. At this time many believed that this method of vaccine treatment was originally intended as a form of specific therapy; but when Kraus and his coworkers in 1917 obtained similar results with colon bacillus vaccine, they opened the door to nonspecific protein therapy in all of its various forms and modifications.

Miller and Lusk in 1916 were the first to use protein as a nonspecific therapy in the United States. They reported favorably on the intravenous use of typhoid vaccine in arthritis.

Vaughan in 1916 maintained that the fever produced by protein therapy was due to the parenteral destruction of the induced protein. He pointed out that the parenteral introduction of bacteria in any form was followed by fever and that fever was produced by the splitting up of the bacterial protein.

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## REFERENCES

- Barbour, H G Proc Soc Exper Biol & Med 18 186, 1921  
 Bowman, K M, and Krasavin, J Arch Neurol & Psychiat 21 2, 1929  
 Cajon, F A, Crouter C Y and Pemberton, R J Biol Chem 57 217, 1923  
 Cecil, R L J A M A 105 1852, 1935  
 Craig, R N Lancet 1 925 1927  
 Dessy, S, Gripiolo F L and Fossati V Notis exper y clin Semin med 21 357, 1914  
 Driver, J R and Shaw H C J A M A 101 26 1931  
 Hildane, J B S and Priestley J G J Physiol 32 252 1905  
 Hill, L and Fluck, M J Physiol 38 1909 Proc the Physiol Soc 3 27, 1909  
 Hoff, Ferdinand Blut und Vegetative Regulation, Ergebn d inn Med u Kinderh 33 195, 1928 Vegetatives Nervensystems und Blut in Lebensnerven und Lebensstriebe  
 Ed by Muller Berlin 1931  
 Hopkins, H Arch Neurol & Psychiat 31 3, 1934  
 Ichikawa, S Ztschr f Immunitätsforsch u exper Therap 23 32, 1914  
 Iskowit, S Acta med Scandinav 82 2 1934  
 Kirby, G H State Hosp Quart 11 1926  
 Kraus, R, and Mazza, S Deutsche med Wchnschr 40 1556, 1914  
 Kraus, R, Penni, J and Bonorino C J Wien. klin Wchnschr 30 868, 1917  
 Labin, B Compt rend Soc de biol 96 1 1927  
 Miller, I L and Iusk F B J A M A 66 1756, 1916  
 Nelson M O Am J Syph 15 185 1931  
 Rothschild, D and Wlamud W Arch Neurol & Psychiat 26 4, 1931  
 Schleicher, E M Am J Clin Path 3 375 1933  
 Torres, E R Vaccinoterapia o intigenoterapia antifica, Semin med 22 1557, 1913  
 Vaughan, V C J Lab & Clin Med 2 15 1916  
 Von Jauregg, Ritter Deutsche Ztschr f Nervenhilk 11, 1926  
 Winslow, C A Miller J A and Noble W C Proc Soc Exper Biol & Med 13 93, 1916  
 Whitehorn, J C Year Book of Neurology Psychiatry and Endocrinology, 1934

## CHLORINE ION DETERMINATIONS ON VENTRICULAR FLUIDS, SUPPLEMENTED WITH A FEW CISTERNAL AND SPINAL FLUIDS IN COMPARISON WITH THE CORRESPONDING BLOOD SERUM\*

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THE chlorine ion concentration of spinal fluid obtained by lumbar puncture has been the subject of numerous studies, while other fractions of the cerebro spinal fluid have been examined only a few times

Cestan, Riser and Laborde have analyzed one single specimen of ventricular fluid, otherwise such analyses have been reported only by Fremont Smith, Mary Dailey and others, who in their large material of spinal fluid examinations have 7 cisternal fluids and 6 ventricular fluids which showed the same chlorine ion concentration as was found in caudal portions of the cerebrospinal fluid

The findings here presented form a part of a series of examinations aiming at a comparison of the concentration of various substances in the ventricular fluid and the corresponding blood serum, supplemented with a few specimens of spinal fluid withdrawn for encephalography and with two cisternal fluids The blood is taken from a median vein of the arm, under paraffin, in the usual manner

\*From the Department of Neurology and Neurosurgery, Rigshospitalet (Chief Professor Viggo Christiansen M D) and the Department of Psychiatry, Rigshospitalet (Chief Professor Aug Wimmer M D)

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## THE EFFECT OF FEVER THERAPY ON THE LEUCOCYTES

After the patients responded to the fever therapy, six hundred white blood cell counts, differential counts, and Arneth counts were done at various time intervals. Standard methods were used for the white blood cell counts and differential counts. The figures under the Arneth count represent the average percentage of lobes present in the polymorphonuclears. The figure 1.86 would indicate a shift to the right, denoting a mature neutrophile, while 1.38 would indicate a shift to the left which is indicative of the proliferation of juvenile neutrophiles. Our normal was established on twenty individuals with normal white and red blood cell concentrations to be 1.70.

TABLE I

THE AVERAGE VALUES OF SIX HUNDRED WHITE BLOOD CELL COUNTS, DIFFERENTIAL COUNTS AND ARNETH COUNTS TAKEN AT VARIOUS TIME INTERVALS

	W.B.C.	PER CENT OF NEUTROPHILES	ARNETH COUNT
One hour before therapy	8,500	58.6	1.86
One hour after therapy	6,931	53.1	1.88
Two hours after therapy	11,891	83.0	1.59
Three hours after therapy	19,684	93.7	1.38
Six hours after therapy	11,042	75.3	1.57
Twenty-four hours after therapy	9,300	64.0	1.66

## THE EFFECT OF FEVER THERAPY ON THE RETICULOCYTES

Schleicher in 1933 reported a new method of counting reticulocytes which seems, for all clinical purposes, to be quite satisfactory. Schleicher found, after a careful study of several hundred smears made from normal human bloods, showing balanced red blood cell and hemoglobin concentrations, the normal range of reticulocyte concentration to be from 2 to 3 per cent. The limits of error in the method employed were plus or minus 1 per cent. In his article he gives a detailed account of the stains and method of enumeration. We utilized Schleicher's method for the determination of the number of reticulocytes present in the patient's blood at the various time intervals while undergoing fever therapy.

TABLE II

THE AVERAGE VALUES OF ONE HUNDRED AND FIFTY RETICULOCYTE COUNTS TAKEN AT VARIOUS TIME INTERVALS

One hour before therapy	2.37 per cent
One hour after therapy	1.76 per cent
Two hours after therapy	2.57 per cent
Three hours after therapy	6.35 per cent
Six hours after therapy	5.34 per cent
Twenty-four hours after therapy	2.72 per cent

## THE EFFECT OF FEVER THERAPY ON THE BLOOD SUGAR LEVELS

A survey of the literature bearing on the problem of the blood sugar level of individuals receiving thermotherapy is rather limited. However, volumes have been written on the various phases of blood sugar in both health and disease. The phase that we are most interested in is the blood sugar levels in mental conditions including the emotional disorders.

TABLE II

CHLORIDE ION CONCENTRATION IN CISTERNAL AND SPINAL FLUIDS AND SERUM

CASE	DIAGNOSIS	I B C	W B C	FLUIDS	SERUM	QUOT
C1*	Supracellar dermoid	50/3	24/3	429.7	363.2	118
C2*	Traumatic atrophy of the brain	6/3	13/3	434.8	357.0	122
L1	Traumatic atrophy of the brain	14/3	2/3	439.4		
L2	Suspicion of intracran tumor	20/3	8/3	434.5		
L3	Suspicion of intracran tumor	9/3	1/3	430.9	355.6	121
L4	Suspicion of intracran tumor	7/3	3/3	438.0		
L5	Suspicion of intracran tumor	800/3	3/3	440.1	370.4	120
L6	Suspicion of intracran tumor	24/3	80/3	442.1		
L7	Glioma of hemi-ph	75/3	6/3	442.0	352.8	125
L8	Suspicion of intracran tumor	60/3	5/3	445.7	369.2	121

\*Cisternal fluids

the cerebrospinal fluid, being about 20 per cent higher than the corresponding value for serum

The material for the analyses here reported was placed at my disposal from the Department of Neurology and Neurosurgery of the Rigshospital. I wish to give my thanks to Professor Viggo Christiansen and Dr. E. Busch for this material.

The analyses were carried out in the laboratory of the Department of Psychiatry, Rigshospitalet. I am greatly indebted to Professor Wimmer for his kind permission to work in the laboratory.

## REFERENCES

Fremont Smith, Mary Duley, and others. Arch. Neurol. & Psychiat. 25: 1271, 1931.  
Rehberg, P. Brandt. Biochem. J. 20: 483, 1926.

## AN OUTBREAK OF FOOD POISONING PROBABLY DUE TO STAPHYLOCOCCUS AUREUS\*

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ON Nov. 17, 1935, two score people were involved in a food poisoning outbreak in this city following the eating of cream puffs with custard filling. It was found that only the people who ate the cream puffs became ill. Other cream puffs from the same lot and coming from the same source were submitted to our laboratory for bacteriologic examination, the day after the food poisoning occurred. A direct microscopic examination showed the presence of a coccus and a large gram positive spore forming bacillus. Some of the filling from four cream puffs was planted, respectively, in four broth tubes. These were incubated at 37° C. for twenty-four hours and a loopful of each was spread over blood serum plates. Two types of colonies were fished to agar slants and after growth were examined morphologically. The yellow colonies proved to be staphylococci and the colorless colonies spore bearing bacilli. The staphylococcus was run through the sugars ordinarily used for its differentiation. It gave the

\*From the Bureau of Laboratories, Department of Health.  
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In order to determine the limits of the blood sugar concentration in patients not undergoing fever therapy, a group of 10 patients were used as controls. This group were given the same breakfast but no fever therapy.

Chart 1 shows the average values of 120 blood sugar determinations at the various time intervals on 30 patients undergoing fever therapy. Superimposed thereon are the average values of 40 blood sugar determinations at various time intervals on 10 patients without fever therapy.

#### THE EFFECT OF FEVER THERAPY ON THE BLOOD PRESSURE, PULSE AND RESPIRATION RATES

Whitehorn (1934) states that in recent studies on the heart in emotional reactions, he has encountered some evidence that the cardiac accelerations associated with brief emotional reactions may be negotiated through central inhibition of the vagus, rather than through sympathetic activity. From the standpoint of its value as a laboratory indicator, the heart is a sensitive indicator of the emotions, even when they are slight and brief, whereas the blood sugar has no practical value as an indicator, even in the most extreme emotional excitement; but this seems to be contrary to other findings.

TABLE III

THE AVERAGE VALUES OF 182 SYSTOLIC BLOOD PRESSURES TAKEN AT VARIOUS TIME INTERVALS ON PATIENTS WHO WERE REACTING TO FEVER THERAPY

Before fever therapy	115 mm. Hg
20 minutes after	103 mm. Hg
40 minutes after	100 mm. Hg
60 minutes after	102 mm. Hg
80 minutes after	109 mm. Hg
100 minutes after	110 mm. Hg
120 minutes after	104 mm. Hg
140 minutes after	106 mm. Hg
160 minutes after	104 mm. Hg
180 minutes after	103 mm. Hg
200 minutes after	102 mm. Hg
220 minutes after	107 mm. Hg
240 minutes after	110 mm. Hg

With Whitehorn's statement in mind, we made a careful investigation of the temperature, pulse, and respiration of patients while they reacted to fever therapy. The temperature, pulse, and respirations were recorded by registered nurses.

TABLE IV

THE AVERAGE VALUES OF 230 TEMPERATURES, PULSES AND RESPIRATIONS TAKEN AT VARIOUS TIME INTERVALS DURING FEVER THERAPY

	TEMPERATURE	PULSE	RESPIRATION
One hour before therapy	98.0° F.	80.5	16.8
One hour after therapy	96.9° F.	79.0	16.7
Two hours after therapy	100.2° F.	98.5	23.8
Three hours after therapy	100.7° F.	102.9	26.6
Six hours after therapy	98.6° F.	87.5	19.9
Twenty-four hours after therapy	98.1° F.	80.0	17.3

When a patient is responding to fever therapy, there is a hyperventilation of the lungs in an attempt to cool the body. This has the effect of removing

## FOLLICULAR LYMPHOBLASTOMA\*

WITH BRIEF REVIEW OF LITERATURE

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IN THE year 1925, Brill, Baehr and Rosenthal of Mount Sinai Hospital, New York, reported their observations on a condition, believed not to have been described previously, which they designated as "generalized giant lymph follicle hyperplasia of lymph nodes and spleen."

Three patients, all women, had been considered. The first patient died following splenectomy, and no autopsy was obtained. The second and third patients were alive and in apparent good health at the time the report was made. The spleen of one was removed, but the other was treated entirely by roentgen ray with gratifying results. The material which underwent pathologic examination consisted of the two removed spleens, with two intraabdominal lymph nodes removed at the same time, and in addition cervical and axillary lymph nodes which were obtained for biopsy from the surviving patients. It was observed that all the lymph nodes throughout the body were enlarged.

In this first presentation the investigators stated that the hyperplastic process was "apparently benign." But in 1927, Baehr and Rosenthal came before the American Association of Pathologists with a further report on the same condition, which they now termed malignant lymph follicle hyperplasia. Again, in 1931, Baehr and Rosenthal, this time in collaboration with Paul Klemperer, before the same society, once more discussed the condition, stating, "The disease is a form of lymphosarcoma which deserves to be distinguished as a pathologic entity because of its characteristic pathology, its unique pathogenetic evolution and its unusual duration. It may form a connecting link between the systemic hyperplasia of the lymphatic tissue and lymphosarcomatosis." To distinguish it from other varieties of lymphosarcoma, it was proposed to term it "follicular lymphoblastoma."

### CASE REPORT

Mrs. W., a white, married female, sixty-seven years of age, entered the hospital on April 18, 1935, with the chief complaint of swelling of the left side of the neck.

Four months ago, patient first noticed swelling in the left supraclavicular region which increased in size very gradually. About a week ago, she noticed swelling along the anterior edge of the left axilla. This swelling did not vary in size under any conditions. It had never been painful.

Patient had no other complaint and stated that she had always been unusually well. Her average weight was about 164, which was high for her height. In her previous medical

\*From the Mountainside Hospital Medical and Pathological Departments, Montclair, New Jersey.

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TABLE V

THE AVERAGE VALUES OF THE TEMPERATURES, PULSES AND RESPIRATIONS TAKEN DURING THE FEVER CRISIS OF THE DIVIDED DOSE METHOD

	TEMPERATURE	PULSE	RESPIRATION
One hour before therapy	98.3° F.	73.2	16.6
First fever crisis	101.3° F.	96.2	23.2
Second fever crisis	104.3° F.	114.5	29.0

TABLE VI

THE AVERAGE VALUES OF THE WHITE BLOOD CELL COUNTS, DIFFERENTIAL COUNTS AND ARNETH COUNTS TAKEN DURING THE FEVER CRISIS OF THE DIVIDED DOSE METHOD

	W.B.C.	PER CENT OF NEUTROPHILES	ARNETH COUNT
One hour before therapy	7,950	55.6	2.06
First fever crisis	18,287	94.6	1.40
Second fever crisis	18,500	95.6	1.37

## COMMENTS

At the present time, all methods of fever therapy are being studied from every available angle. Some investigators believe that the hyperpyrexia is the sole factor in bringing about good results. Others raise the question that if the hyperpyrexia is the sole factor, how can they account for the fact that more uniform results are not obtained. Among our group there were seven patients who did not respond to the typhoid-paratyphoid vaccine. They manifested no clinical symptoms and no changes were noted in their blood laboratory work.

We have found, using the single dose method, that within the first hour after the typhoid-paratyphoid vaccine has been administered the patient experiences a state of shock, accompanied by nausea and chills; also some cases become quite cyanosed. During the period of shock a marked leucopenia, a slight shift to the left in the Arneth count, no change in the reticulocyte count, a hypoglycemia and a decrease in the systolic blood pressure occur.

Shortly after the state of shock (from two to three hours after the therapy), the patient develops a moderately high fever. During this hyperpyrexia the leucopenia is replaced by a leucocytosis. At this time a decided shift to the left in the Arneth count, a marked increase in the reticulocytes, an increase in the blood sugar level and an increase in the systolic blood pressure occur.

Ferdinand Hoff (1931) has laid down the law that vagotonic or, more properly, parasympatheticotonic conditions incline to leucopenia, a lymphatic tendency and eosinophilia. Sympatheticotonic conditions on the other hand incline to leucocytosis, a myeloid tendency with a left shift of the nuclear count and a lowering of the eosinophiles.

If this law may be taken as a guide we may deduce the conclusion from our results that bacterial protein fever therapy has resulted in a primary parasympathetic stimulation, followed by a more marked and prolonged sympathetic stimulation.

The authors wish to express their gratitude to Thomas Verner Moore, for his cooperation and helpful suggestions in completing this study.

8,000. Polynuclears averaged about 64 per cent, lymphocytes about 35 per cent, and the eosinophiles 1 per cent. In the morphologic study of the red blood cells, an occasional microcyte was found.

*Urinalysis.*—

Color—yellow, clear	Sugar—negative	Indican—negative
Sp. Gr.—1.015	Protein—negative	
Reaction—acid	Acetone—negative	

Microscopic: Moderate number of squamous epithelial cells. A few large, round, epithelial cells, 13 WBC/HPF

On re examination on April 21, 1936, after extensive therapy, her cardiovascular condition was improved so that the pretibial pitting disappeared. The slight shortness of breath was relieved. The blood pressure fell and has never returned to the original level. The last reading on the twenty first of April, 1936, was 140/60

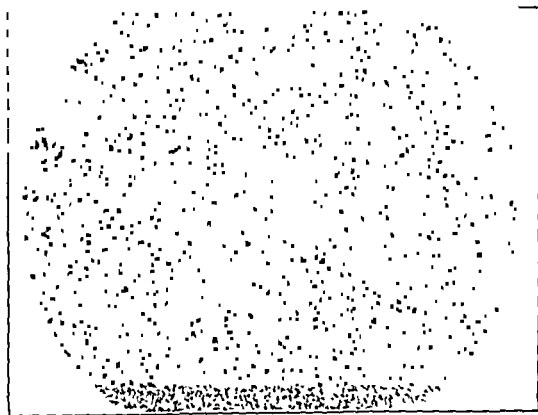


Fig. 2—Low Power Individual follicles occupy several microscopic fields Notice the absence of intervening tissue

Throughout her illness neither liver nor spleen could be palpated on frequent examinations. It was not until December, 1935, that the liver came just to the edge of the costal margin on deep inspiration, and in the right lateral recumbent position, the edge of the spleen could be palpated. It was not easily palpable in the dorsal recumbent position. It was not tender. The spleen has remained palpable ever since, although changing practically not at all in size

The treatment consisted of deep x ray therapy with iron and arsenic by mouth because of the slight tendency to anemia. From time to time she was given arsenic alone by mouth. The cervical and axillary lymph nodes were removed on April 18, 1935, and submitted to the laboratory for examination

#### PATHOLOGIC REPORT

##### *Cervical and Axillary Lymph Nodes*

*Macroscopic Examination*—Many lymph nodes were submitted for examination, which weighed collectively 180 gm, and on cut section, the capsules appeared to be intact and unin-

The analyses are carried out after the method given by Rehberg (a modification of Van Slyke's method). The principle of this technic is: 0.1 c.c. of serum or spinal fluid is mixed with 0.5 c.c. of 0.03 N solution of  $\text{AgNO}_3$  and boiled in a water-bath for half an hour, with addition of perhydrol. After cooling, 1 as an indicator. The Rehberg microburette is employed in the titration. The c.c. of ether is added, and titration is performed with 0.1 N ammonium thiocyanate, with air being led through the tube and with ferric ammonium sulphate as an indicator. The Rehberg microburet is employed in the titration. The calculations are performed according to known solutions of NaCl.

Triple analyses are made, and the mean value is calculated therefrom. Only in exceptional instances is the experimental error of the individual analyses greater than 4 mg. per cent.

The tables give the preliminary results. The third and fourth columns give the results of the cell counts in 3 c.mm. which have always been made within one hour after withdrawal of the fluids.

The fifth and sixth columns give the chlorine ion concentration in milligram per cent. The last column gives the quotient (spinal fluid chlorine divided by serum chlorine).

Analyses of 30 ventricular fluids, supplemented with 8 spinal fluids and 2 cisternal fluids, show that the chlorine ion concentration is the same throughout

TABLE I  
CHLORINE ION CONCENTRATION IN VENTRICULAR FLUIDS AND SERUM

CASE	DIAGNOSIS	R.B.C.	W.B.C.	VENTR. FL.	SERUM	QUOT.
1	Glioma of hemisph.	400/3	6/3	413.2	355.0	116
2	Suspicion of intracran. tumor	100/3	4/3	448.2	356.6	126
3	Spongioblastoma of hemisph.	400/3	6/3	445.6	374.5	119
4	Suprasellar dermoid	210/3	60/3	441.6	375.9	117
5	Suspicion of intracran. tumor	140/3	4/3	436.3		
6	Taberous sclerosis	4500/3	35/3	437.9	358.1	122
7	Ependymitis	30/3	2/3	443.8	366.5	121
8	Suspicion of intracran. tumor	202/3	10/3	439.5	370.7	119
9	Suspicion of intracran. tumor	80/3	11/3	439.5	368.7	119
10	Ependymitis	290/3	4/3	447.3	372.8	120
11	Suspicion of intracran. tumor	1180/3	12/3	438.6	364.6	120
12	Suspicion of intracran. tumor	394/3	18/3	436.1		
13	Parasagittal meningioma	900/3	5/3	438.6	364.6	120
14	Glioma of the brain stem	680/3	25/3	441.1	366.7	120
15	Glioma of hemisph.	25/3	75/3	435.4	361.1	121
16	Glioma of hemisph.	98/3	6/3	450.1	356.8	126
17	Suspicion of intracran. tumor	140/3	16/3	457.6		
18	Venous aneurysm	12/3	6/3	462.4	373.5	124
19	Glioma of hemisph.	18/3	4/3	436.0		
20	Meningioma of petrous bone	340/3	5/3	468.1	388.7	120
21	Suspicion of intracran. tumor	356/3	4/3	456.8	372.8	123
22	Suspicion of intracran. tumor	400/3	210/3	455.0	381.4	119
23	Suspicion of intracran. tumor	216/3	3/3	451.6	377.1	120
24	Traumatic lesion of hemisph.	1680/3	5/3	448.2	366.5	122
25	Meningioma of convexity	550/3	12/3	449.5	368.2	122
26	Glioma of hemisph.	1150/3	18/3	457.5	370.5	123
27	Glioma of hemisph.	2500/3	6/3	448.2	365.9	122
28	Suspicion of intracran. tumor	620/3	2/3	451.0	374.4	120
29	Suspicion of intracran. tumor	60/3	1/3	452.2	373.6	121
31	Glioma of hemisph.	950/3	26/3	453.7	370.7	122

8,000. Polynuclears averaged about 61 per cent, lymphocytes about 35 per cent, and the eosinophiles 1 per cent. In the morphologic study of the red blood cells, an occasional microcyte was found.

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Fig. 2.—Low Power. Individual follicles occupy several microscopic fields. Notice the absence of intervening tissue.

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#### PATHOLOGIC REPORT

##### *Cervical and Axillary Lymph Nodes*

*Macroscopic Examination*—Many lymph nodes were submitted for examination, which weighed collectively 180 gm, and on cut section, the capsules appeared to be intact and unim-

expected reactions in all but one sugar—it did not ferment lactose. It liquefied gelatin and produced hemolysis on blood agar plates.

Aside from ruling out the anthrax bacillus, nothing further was done with the spore-forming bacillus, as we know of no instance in which organisms of the aerobic spore-forming group of bacilli (*B. subtilis* group) have produced food poisoning. Further studies in our laboratory have shown that the *B. subtilis* group appears notoriously as contaminants in custard fillings. For toxin determination, twenty-four-hour broth cultures of the staphylococcus were inoculated intraperitoneally into a white mouse and a guinea pig, and intravenously into a rabbit. There were no particularly noticeable effects in the mouse or guinea pig. The rabbit, although showing symptoms of toxemia at the end of three hours, recovered rapidly and was alive at the end of one month.

The staphylococcus isolated from the custard filling in question was a single strain. It produced a golden yellow pigment. The morphology was that of a typical gram-positive coccus occurring in clumps. Dextrose, maltose, saccharose, and mannitol produced acid but no gas. Lactose, raffinose, inulin, and salicin were not affected. Immunologic reactions were not used in identification.

Since the custard filling which contained the staphylococcus was heated, it seemed desirable to determine whether the organism was especially heat resistant. A broth culture in milk withstood a temperature of 70° C. (158° F.) for one-half hour. The organism probably would have been more heat resistant in the custard filling because of its thickness.

We are inclined to believe that the filling was contaminated after it was heated rather than that the organism survived the heat exposure to which it was subjected, although we think this latter quite probable.

As the symptoms appeared within three or four hours after ingestion of the cream puffs, we think the outbreak of food poisoning must be considered to be almost certainly due to a bacterial toxin or to a formed toxic substance produced by the *Staphylococcus aureus* present in the filling. We had neither facilities nor opportunity to prove this strain of *Staphylococcus aureus* to be of the food poisoning type either by monkey feeding experiments or by tests on human volunteers.

An epidemiologic investigation was made of the personnel in the bakery where the cream puffs were made. No open suppurative lesions were found. Routine cultures were taken from the nose and throat of each person employed. *Staphylococcus aureus* was found in pure culture in the throat of one of the men and showed the same morphologic, cultural, biochemical, and physiologic characteristics as the strain isolated from the custard filling of the cream puffs. It was later learned that this man had so-called grip and a sore throat at the time this culture was taken. A second specimen from this man also showed the presence of a pure culture of *Staphylococcus aureus* identical in all characteristics with the strain isolated from the first culture and also with the strain isolated from the custard filling. We think the repeated recovery of *Staphylococcus aureus* in pure culture from the throat of this man incriminates him as the original source of the etiologic agent.

Thus by 1933 the efforts of pathologists had placed before the medical profession a fairly clear picture of a condition previously shrouded in a mist of clinical confusion. Much obscurity still clouds this picture, etiology, pathogenesis, and clinical symptoms must still be cleared up by the slow accumulation of data supplied by individual practitioners. It is, therefore, of importance that all such data should be put on record. The present paper is an effort in that direction.

It is Klemperer's belief that in follicular lymphoblastoma we have a link between lymphadenosis and lymphosarcomatosis. "While early phases resemble aleucemic lymphadenosis, necropsy *versus* *litera* presents evidence of tumor formation of lymph nodes and invasion of neighboring structures, precisely as in lymphosarcomatosis. One of the cases of follicular lymphoblastoma developed a blood picture characteristic of chronic lymphatic leukemia. At necropsy, all of the lymph nodes were found to be enlarged. Other autopsied cases conformed to the anatomic picture of lymphosarcomatosis because of conspicuous aggressive tumor formation of the lymph nodes with infiltration of the neighboring organs. In the early stages of its evolution, the process is merely hyperplastic, while in the later stages it becomes atypical and aggressive. All such experiences point to a close affinity between the simple hyperplastic and the neoplastic proliferations of the lymphatic system. This is evident even in the spleen, the appearance of which has hitherto constituted one of the chief reasons for strict separation. Whenever the spleen is involved in Hodgkin's disease, the alteration is macroscopically characteristic, and can be easily differentiated from the splenic lesions observed in the hemoblastoses." In this investigator's opinion, Hodgkin's disease is essentially a granulomatous process, in which the appearance of the spleen differs fundamentally from that seen in the hemoblastoses.<sup>4</sup>

The diagnostic difficulties which may arise in the attempt to differentiate between Hodgkin's disease and lymphosarcoma are, in Klemperer's opinion, due to undifferentiated proliferation of the reticulum of the lymphoid tissues. The changes in the follicles he believes to be proliferation of embryonal elements which under suitable stimuli can proliferate and differentiate in the same manner as other embryonal elements placed elsewhere in the body. If we were to employ a "noncommittal term" (he suggests "progressive reticulum cell proliferation"), false interpretation of the transitions between the individual fixed groups, such as lymphosarcoma, Hodgkin's disease and so on, might be avoided.

It might perhaps be well, before proceeding to a description of individual cases, to refresh our memories as to just what is meant by the term "lymphoblastoma," and estimate, even if we cannot clear away, the ambiguities which have in the past surrounded, and still surround, any consideration of this branch of the great family of entoplastic growths. Lymphoblastoma is defined by Mallory<sup>6</sup> as a tumor of mesodermal origin, the cells of which tend to differentiate into lymphocytes—that is, cells of the lymphocyte series. As the lymphoblast, which is the type cell, under normal conditions may differentiate first into lymphocyte, and thence to what we term a "lymphoid cell," it is not difficult to foresee that, under pathologic conditions, such changes may take place with far greater rapidity and variation than is usual in health.

history the only diseases which she recalled were scarlet fever which she had had in early childhood and some indefinite fever which was called malaria. Her family history was negative as far as any glandular enlargements were concerned and also negative as regarded any ordinary chronic illnesses. Her father died at ninety one, and her mother died at ninety-six.

*Physical Examination.*—

1. General Appearance: Short, stout, somewhat dark pigmentation of skin; suggested hypothyroid type.
2. Eyes: Pupils equal and reacted to light.
3. Mouth: No abnormality of mouth or teeth.
4. Throat: Negative.
5. Neck: In the left supraclavicular space toward the neck was a mass estimated to be between 3 and 4 cm. in diameter, firm, no fluctuation, not easily movable, not tender.

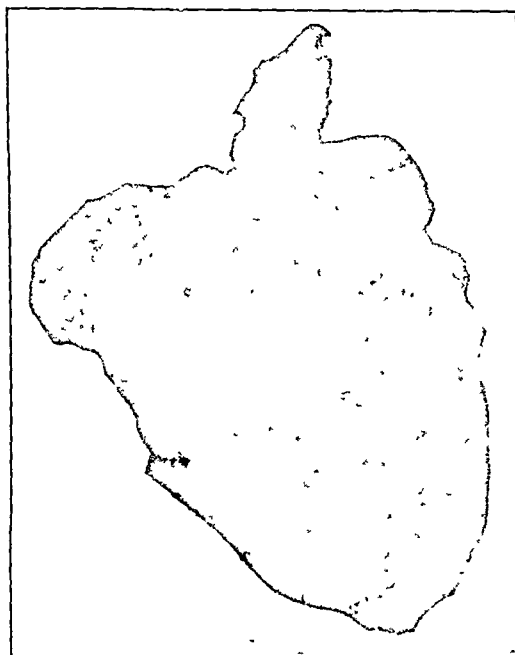


Fig. 1.—Note the prominent follicles and bulging of tissue.

6. Heart: Very moderately enlarged to the left by percussion. Left border 12 cm. from the midline. Right border was just about under the right costal margin. Rhythm regular. Rate 82. Blood pressure 188/106, left arm, sitting. Rough systolic murmur at aortic area.
7. Lungs: A few râles at the left base posteriorly.
8. Abdomen: Liver not palpable. Spleen not palpable. Abdomen otherwise negative.
9. Extremities: No axillary glands palpable. Very slight pretibial pitting in the lower portion of the leg.

*Radiographic Examination of the Chest.*—Films of the chest revealed no evidence of enlarged mediastinal glands. Marked hilus thickening and increased peribronchial fibrosis were seen. There were only occasional enlarged bronchial nodes seen in the hilus. Lung fields were clear of any infiltration.

*Blood Counts.*—Numerous blood counts were done, and the hemoglobin averaged from 14.3 gm. to 12.4 gm., and the red blood cells about 4,730,000 and white blood cells about

grounds there may be objections to the generic name 'lymphoblastoma' it can answer the purpose for which it is intended, at least until more is known in regard to the etiologic factors "

One hundred and fifty six cases of lymphadenopathy were studied at Mount Sinai Hospital, New York, during a ten year period. Of these, 80 were Hodgkin's disease, 50 were lymphosarcoma, 16 were reticulum cell sarcoma, and only 10 were diagnosed as follicular lymphoblastoma. It was upon the findings in these 10 cases that the reports from this hospital cited earlier in this paper, were made. In 1931 Rosenthal, assisted by Harris and Kean, made still another report, this time giving special attention to the pathologic, clinical and radiotherapeutic features. A thorough review of the literature fails to reveal any comprehensive report on the clinical manifestations or radiosensitivity of this subvariety of lymphosarcoma "

If a biopsy is done in the early stage of this disease, enormously enlarged lymphoid follicles are found which resemble markedly overgrown germinal centers. These follicles are composed of lymphoblasts and frequently show mitotic figures. The periphery of the follicle is composed of the small lymphocytes with darker staining nuclei. These morphologic and histologic findings are characteristic and are of especial interest now when so much stress is being placed upon the grading of tumors, with its bearing on radiosensitivity and prognosis.

Follicular lymphoblastoma may be mistaken for simple follicular hyperplasia. However, careful study readily differentiates the former by the presence of mitotic figures, by the large size of the follicles, and by the tendency to involvement of the fibrous capsule. The tissue does not resemble Hodgkin's disease histologically. It may be differentiated from aleucemic lymphadenosis by the remarkable follicular hyperplasia.

In a later stage of this disease, in addition to the characteristic follicular condition, some areas may show a disruption of this formation, and a rather typical picture of lymphosarcoma with infiltration of the capsule and, at times, invasion of surrounding structures. The spleen, which is usually greatly enlarged, shows a similar characteristic follicular hyperplasia.

It was the experience of these investigators that the duration of the affection was exceptionally long for disease having such characteristics. One of these patients is still alive after fourteen years. In all ten patients the first indication was enlargement of some group of nodes, cervical, axillary or inguinal. "Terminally, the body may be riddled with disease, voluminous tumor deposits occurring which give rise to pleural effusion and ascites." Four patients, all women, developed exophthalmos.

#### TREATMENT

While no form of treatment has yet been devised which will offer permanent relief in any of the class of interrelated diseases which we now term "lymphoblastoma," the growths have all proved radiosensitive, and clinical improvement usually follows judicious x-ray therapy. No physician is justified in holding out ultimate hope to the patient or his friends, but the long survival of some

The lymphoblastoma is an infiltrative tumor, therefore its stroma is furnished by the organ or tissue that it invades. In some tumors the stroma is of the slightest, consisting chiefly of capillary blood vessels accompanied by a minimum of connective tissue, usually in the form of a reticulum. As the invaded organs enlarge, the stroma may be increased by proliferation of fibroblasts and probably by formation of new blood vessels. The lymphoblastoma probably originates from a single cell—a lymphoblast. Cells of this type occur in nearly all parts of the body, but are most common in the various lymph nodes, and in the lymph nodules of the gastrointestinal tract and spleen. On this account the lymphoblastoma may originate in various localities wherever lymphoid tissue exists, as, for example, in the cecum or spleen, but it starts most commonly in lymph nodes, especially in those of the cervical, axillary and inguinal regions, and in the mediastinum.

Discussions of the limitations of the term "lymphoblastoma" have not been lacking. Keim<sup>7</sup> lists no less than fifteen conditions which are so essentially similar as to justify (in his opinion at least) their inclusion under the single classification of "lymphoblastomas." The dermatologic aspect of the condition has received particular attention, and contributions regarding manifestations upon the cutaneous surface are of great interest and importance. But the similarity or identity of the skin conditions to the follicular type is still being questioned in some quarters. "True lymphadenotic infiltration of the skin occurs most commonly in association with the forms of lymphatic hyperplasia, usually referred to as leukemia, lymphosarcoma, granuloma fungoides and Hodgkin's disease, as well as certain transitional forms originally described as Sternberg's leukosarcoma and kaposi lymphoderma perniciosum." Fraser<sup>8</sup> more than a decade ago called attention to the relationship which exists between mycosis fungoides, lymphatic leucemia and lymphosarcoma. Similar views were held by Douglas Symmers a number of years previously, for he wrote in 1918,<sup>9</sup> "Chronic lymphatic leukemia and its companion lesion, pseudo-leukemia, together with the familiar examples of lymphosarcoma, present many clinical and anatomic changes in common. The histologic alterations are closely akin; in fact, in many instances, they are indistinguishable one from another." Mallory's classification offers a simple way out of such difficulties as Symmers visualizes, that is, the grouping of all such lesions under the general heading of lymphoblastomas. Goeckerman and Montgomery<sup>10</sup> have reported a small series of cases of great interest in that they serve to illustrate how many features in common there are between certain vaguely understood skin lesions and the neoplastic growths seen in the lymph nodes and internal organs which are usually designated as lymphosarcomas. In their own words: "A clinical consideration of our two cases leaves little doubt that they belong to the group of lymphoblastomatous diseases, yet they can only with difficulty be placed definitely in subgroups under this heading because they bear none of the classic clinical and histopathologic characteristics of any one disease. In our opinion they favor the view that this entire group is closely related genetically and that it contributes to confusion rather than to clarification to insist on sharp distinctions at all times. A generic term including this entire group seems desirable. Although on etymologic

# THE SIGNIFICANCE OF ABERRANT BASAL METABOLIC TRACINGS\*

## CLINICAL NOTE

HORACE B. CATES, M.D., LOS ANGELES, CALIF.

**E**MOTIONAL instability may at times be revealed by the spirometric tracing used in the determination of the basal metabolic rate by indirect calorimetry. The spirometer records the speed, depth and regularity of the respiratory movements. It has been observed that irregular or sighing types of respiration are frequently found in patients with functional heart disease or with some manifestations of the psychoneuroses. The following note is written in the hope that it will call attention to the possibility of recognizing functional disorders from the inspection of spirometric tracings.

Irratic basal metabolic tracings taken by competent technicians were selected, and the corresponding patients' histories were reviewed. Basal metabolic rate determinations from 400 patients were studied, and 32 were selected as indicating a psychogenic reaction. Seventy five per cent of the selected cases were from patients between the second and fourth decades. Females predominated. Thirty two cases, or 8 per cent, of the records exhibit these bizarre irregularities and most of the histories indicate functional disorders. In general, the selected tracings may be divided into two types. First, those who sigh at intervals, causing exaggerated excursions of the spirometer. In the second type the slope of the tracing is wavelike with alternating crests and troughs.

Nielsen and Roth,<sup>1</sup> reviewing 20,000 basal metabolic tests, divide the tracings into nine types. Six of these respiratory types are found in children. One of their selected spiograms Type G described as "a regular inspiratory line interrupted by frequent and extremely deep inspirations," is akin to the tracings under discussion in this paper. These Type G tracings are most prevalent in females and when once established at puberty do not disappear at menopause. Ziegler and Levine,<sup>2</sup> determining the effects of the emotions on the basal metabolic rate, found the metabolic rate might be altered as much as 35 per cent, and there were observed changes in amplitude and rate as compared with the control tracings. Davis Baker<sup>3</sup> quotes Paul White as having found sighing a common symptom in women of the third and fourth decades with neurocirculatory asthenia but relatively rare in organic heart disease. She concludes that sighing respiration aids in determining the relative degree of responsibility of the heart and nervous system in the production of disability. Craig and White<sup>4</sup> reported on 50 cases of neurocirculatory asthenia without organic heart disease and found that 70 per cent had respiratory disturbance. Christie<sup>5</sup> found the anxiety neuroses produced an irregular shallow type of respiration, while the

\*From the Department of Medicine, University of Southern California.  
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volved. The tissue was solid, pinkish-white in color, and bulged. The follicles were easily detected because of their prominence.

*Microscopic Examination.*—The individual follicles occupied several microscopic fields when viewed with low power. They were so close to each other that there was no intervening pulp visible, and the lymph sinuses were compressed and obliterated. Most of the follicles were composed of an endothelioid type of cell. The active cells with mitoses far outnumbered the pyknotic forms, which formed a dark ring around the margin of the follicle. The endothelioid type of cell was largely polygonal and contained a large amount of cytoplasm.

This lesion was considered benign by many, yet there are cases where they have recurred and finally, after many years, the condition may terminate as a lymphosarcoma. These were radiosensitive.

*Diagnosis.*—Follicular lymphoblastoma of the cervical and axillary lymph nodes.

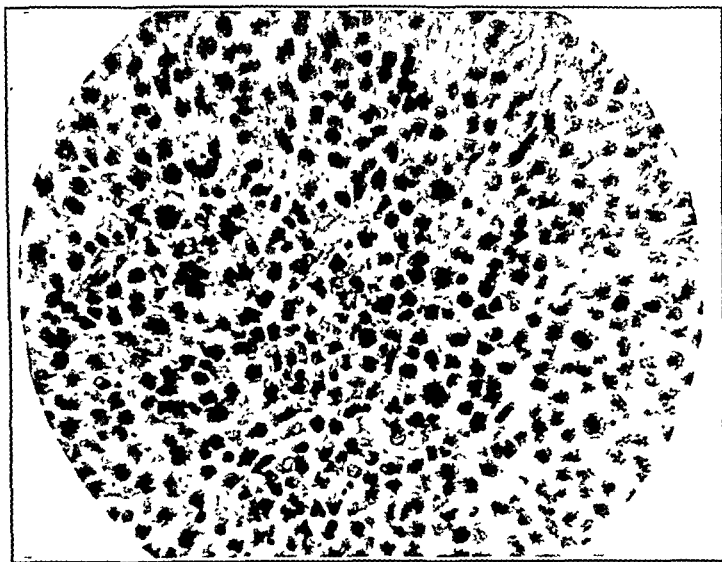


Fig. 3.—High Power. Note active cells with mitoses. The lymphocytes are at the margin of the follicles.

#### DISCUSSION

The salient characteristics of the condition were listed as follows:

1. Lymphadenopathy due to hyperplasia of the germinal centers of the lymph follicles.
2. Splenomegaly due chiefly to enormous enlargement of malpighian bodies, the weight of the spleen increasing up to 1,800 gm.
3. Absence of abnormal cells in the blood.
4. Absence of anemia or cachexia.
5. Tendency to development of serous effusions in the pleural and peritoneal cavities due to pressure of mediastinal or abdominal lymph nodes upon venous or lymph vessels.
6. Absence of involvement of tonsils and lymphatic apparatus of the gastrointestinal tract.
7. Tendency to lymphatic infiltration in lachrymal gland resulting in unilateral exophthalmos. Aside from the absence of anemia and cachexia, the chief differential feature, distinguishing the condition from lymphosarcoma, is its origin multicentrically throughout the body in the lymph follicles, whereas lymphosarcoma arises monocentrically and spreads by lymphatic extension.

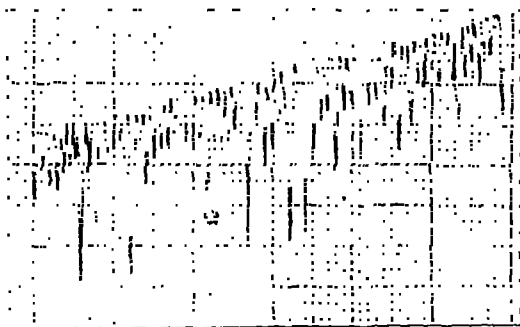


Chart 2

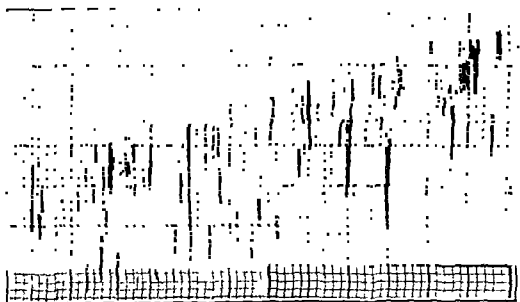


Chart 3.

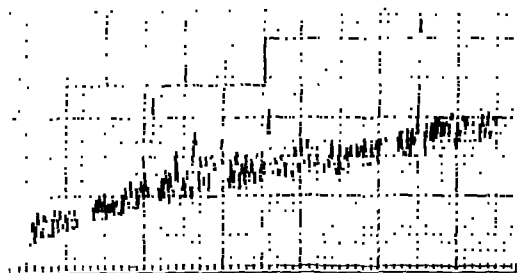


Chart 4.

The lymphoblastoma is an infiltrative tumor, therefore its stroma is furnished by the organ or tissue that it invades. In some tumors the stroma is of the slightest, consisting chiefly of capillary blood vessels accompanied by a minimum of connective tissue, usually in the form of a reticulum. As the invaded organs enlarge, the stroma may be increased by proliferation of fibroblasts and probably by formation of new blood vessels. The lymphoblastoma probably originates from a single cell—a lymphoblast. Cells of this type occur in nearly all parts of the body, but are most common in the various lymph nodes, and in the lymph nodules of the gastrointestinal tract and spleen. On this account the lymphoblastoma may originate in various localities wherever lymphoid tissue exists, as, for example, in the cecum or spleen, but it starts most commonly in lymph nodes, especially in those of the cervical, axillary and inguinal regions, and in the mediastinum.

Discussions of the limitations of the term "lymphoblastoma" have not been lacking. Keim<sup>7</sup> lists no less than fifteen conditions which are so essentially similar as to justify (in his opinion at least) their inclusion under the single classification of "lymphoblastomas." The dermatologic aspect of the condition has received particular attention, and contributions regarding manifestations upon the cutaneous surface are of great interest and importance. But the similarity or identity of the skin conditions to the follicular type is still being questioned in some quarters. "True lymphadenotic infiltration of the skin occurs most commonly in association with the forms of lymphatic hyperplasia, usually referred to as leukemia, lymphosarcoma, granuloma fungoides and Hodgkin's disease, as well as certain transitional forms originally described as Sternberg's leukosarcoma and kaposi lymphoderma pernicioso." Fraser<sup>8</sup> more than a decade ago called attention to the relationship which exists between mycosis fungoides, lymphatic leucemia and lymphosarcoma. Similar views were held by Douglas Symmers a number of years previously, for he wrote in 1918,<sup>9</sup> "Chronic lymphatic leukemia and its companion lesion, pseudo-leukemia, together with the familiar examples of lymphosarcoma, present many clinical and anatomic changes in common. The histologic alterations are closely akin; in fact, in many instances, they are indistinguishable one from another." Mallory's classification offers a simple way out of such difficulties as Symmers visualizes, that is, the grouping of all such lesions under the general heading of lymphoblastomas. Goeckerman and Montgomery<sup>10</sup> have reported a small series of cases of great interest in that they serve to illustrate how many features in common there are between certain vaguely understood skin lesions and the neoplastic growths seen in the lymph nodes and internal organs which are usually designated as lymphosarcomas. In their own words: "A clinical consideration of our two cases leaves little doubt that they belong to the group of lymphoblastomatous diseases, yet they can only with difficulty be placed definitely in subgroups under this heading because they bear none of the classic clinical and histopathologic characteristics of any one disease. In our opinion they favor the view that this entire group is closely related genetically and that it contributes to confusion rather than to clarification to insist on sharp distinctions at all times. A generic term including this entire group seems desirable. Although on etymologic

## A STUDY OF THE RELATION OF RICKETS TO ANEMIA\*

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THE association of anemia with rickets in infancy has been a disputed question among clinicians for many years. Some observers regard anemia as a symptom of rickets and feel that a causative factor is to be found in the changes which occur in the bone marrow in this disease. Hess<sup>1</sup> states that Marfan is one of the foremost of such observers. On the other hand, many believe the anemia in rickets is produced by complicating factors such as poor diet and infection. Findlay,<sup>2</sup> in a study of 30 cases of active rickets, found anemia to be the result of complications in most of the group and discovered that many of his rachitic infants had normal or elevated hemoglobin and red blood cell counts. Griffith and Mitchell<sup>3</sup> state that the hemoglobin of the blood is reduced in severe cases of rickets. Royster<sup>4</sup> has remarked about the frequent association of rickets with chronic nutritional disorders such as lead to secondary anemia. Hess feels that anemia is not a characteristic symptom of rickets but states that, as the disorder advances, it is generally accompanied by anemia of some degree. We have studied a group of thirty infants and children ranging in age from six months to three years, who were admitted to the State of Wisconsin General Hospital with a diagnosis of active rickets. Table I contains a list of these cases divided into the uncomplicated and complicated groups. Of the whole series of cases, suffering from mild to severe acute rickets, 16 or 53 per cent had normal hemoglobin readings and red blood cell counts. Fourteen or 47 per cent had readings of 60 per cent hemoglobin or lower. Eight of the cases showing low hemoglobin readings were complicated by dietary deficiencies or infections. Eleven cases in the entire group were complicated by dietary deficiencies, infections or anomalies such as cleft palate. Eight of the eleven complicated cases showed anemia whereas only six of the nineteen uncomplicated cases revealed a reduction in hemoglobin.

In an attempt to throw some light on the relationship of anemia to rickets, experiments were undertaken to try to produce the picture of rickets with anemia in the chick. This work consisted of the production of severe rickets in the chick and a comparison of the hemoglobin content of the blood of the rachitic with that of the normal chick. Analyses of the livers of normal and rachitic chicks for their total and available iron contents were also made.

### EXPERIMENTAL

One day old white leghorn chicks were used in these studies. They were housed in the usual manner in heated cages on two mesh to the inch wire screens.

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of these patients who were systematically treated by roentgen ray exposures, gives ground for assurance that some aid may be offered. Rosenthal and his coworkers used an average dose of about 200 r., treating all the involved areas at three- or four-day intervals. "The lesions throughout the body responded readily until the terminal stage of the disease, when they became radio-resistant. In several instances, radium packs induced regression when roentgen therapy failed." It was the consensus of opinion among those who discussed the paper just quoted that surgery is of no use in the management of any of these lymph system affections. Herbert Fox of Philadelphia strove to emphasize the differences between the various clinical conditions which we have sought to link together under the common title "lymphoblastoma." He regarded this as important from the point of view of treatment. He defined lymphomatosis as a multiple tumor of lymph nodes and viscera, with or without an enlargement of the spleen. Lymphosarcoma manifests none of the characteristics of lymphomatosis; there is no reduction of neutrophiles as in leucemia; no enlargement of the spleen; no involvement of the skin or the bones; and no distinctive involvement of the lungs. In Hodgkin's disease, on the contrary, though the spleen is often large, it is not characteristically hypertrophied, while involvement of skin, bone, and lungs is very common.

It is to be hoped that further reports of pathologic findings and methods, tried out in treatment, will soon add to our knowledge of this obscure class of neoplastic growths.

#### REFERENCES

1. Brill, N. E., Baehr, G., and Rosenthal, N.: Generalized Giant Lymph Follicle Hyperplasia of Lymph Nodes and Spleen, *J. A. M. A.* 84: 668, 1925.
2. Baehr, G., and Rosenthal, N.: Malignant Lymph Follicle Hyperplasia of Spleen and Lymph Nodes, *Am. J. Path.* 3: 550, 1927.
3. Baehr, G., Klemperer, P., and Rosenthal, N.: Follicular Lymphoblastoma (Giant Follicular Hyperplasia of the Lymph Nodes and Spleen), *Am. J. Path.* 7: 558, 1931.
4. Klemperer, Paul: The Spleen in Hodgkin's Disease, Lymphosarcomatosis and Leukemia, *Am. J. M. Sc.* 188: 593, 1934.
5. Idem: The Relationship of the Reticulum to Diseases of the Hematopoietic System, *Contrib. Med. Sc., Emanuel Libman* 2: 655, 1932.
6. Mallory, F. B.: *The Principles of Pathologic Histology*, Philadelphia, 1914, W. B. Saunders Co., p. 326.
7. Keim, H. L.: The Lymphoblastomas, Their Interrelationships, *Arch. Dermat. & Syph.* 19: 532, 1929.
8. Fraser, J. F.: Mycosis Fungoides: Its Relation to Leukemia and Lymphosarcoma, *Arch. Dermat. & Syph.* 12: 814, 1925.
9. Symmers, Douglas: Certain Unusual Lesions of the Lymphatic Apparatus, *Arch. Int. Med.* 21: 237, 1918.
10. Goeckerman, W. H., and Montgomery, H.: Cutaneous Lymphoblastoma: Report of Two Unusual Cases, *Arch. Dermat. & Syph.* 24: 383, 1931.
11. Rosenthal, N., Harris, Wm., and Kean, Albert: Clinical and Radio-Therapeutic Considerations of Follicular Lymphoblastoma, *Am. J. Roentgenol.* 29: 95, 1933.
12. Fox, Herbert: Discussion of.<sup>11</sup> *Ibid.*, p. 102.

which time the average readings for the normal and rachitic groups were 8.60 gm and 7.64 gm, respectively. The difference of 0.96 gm was not regarded as significant. This experiment was repeated, using groups of 10 chicks each and hemoglobin readings were made in the second and fifth weeks. The average results which were almost identical are recorded in Table II.

TABLE II  
HEMOGLOBIN CONTENT OF THE BLOOD OF RACHITIC AND NORMAL CHICKS  
AVERAGE READINGS IN GRAMS OF HB/100 CC OF BLOOD

AGE IN WEEKS	PART I		PART II	
	NORMAL (15 CHICKS)	RACHITIC (15 CHICKS)	NORMAL (10 CHICKS)	RACHITIC (10 CHICKS)
2	8.45	8.45	8.54	8.42
3	8.02	7.11		
4	8.00	7.98		
5	8.00	7.64	8.14	8.47

In order to determine the amount of total and available iron in the livers of the normal and rachitic chicks the experiment was repeated, using the same method as described above. Each group placed on the experiment contained 10 to 15 one-day-old, white leghorn chicks. Beginning in the fourth week, after signs of well marked rickets had developed several chicks were taken from each group, killed by decapitation, and the livers removed and analyzed for the total and available iron content. The livers were washed thoroughly in distilled water.

TABLE III  
TOTAL AND AVAILABLE IRON CONTENT OF LIVERS FROM RACHITIC AND NORMAL CHICKS

NORMAL CHICK NO	AGE IN WEEKS	WEIGHT IN GRAMS	WEIGHT OF FRESH LIVER	AVAILABLE IRON IN MG/GRAM OF LIVER		TOTAL IRON IN MG/GRAM DRY LIVER	PERCENTAGE OF AVAILABLE IRON
				FRESH	DRY		
NORMAL							
4173	4	208	6.1447	0.029	0.117	0.25	46
4174	4	209	6.8688	0.0275	0.110	0.179	61
4141	5	293	8.1043	0.0275	0.110	0.23	48
4142	5	294	9.1507	0.028	0.112	0.282	40
4144	6	272	8.8043	0.0215	0.0855	0.187	45
4149	6	270	11.4964	0.035	0.140	0.22	64
4146	8	460	14.5161	0.0242	0.0968	0.20	48
4147	8	367	9.0451	0.039	0.156	0.316	49
4150	8	385	11.3351	0.029	0.116	0.232	50
Average				0.0291	0.116	0.232	50
RACHITIC							
RACHITIC							
4131	4	130	2.3517	0.0348	0.134	0.24	56
4133	4	120	3.7635	0.0382	0.157	0.33	46
3592	4 (D)	130	4.1789	-	-	0.50	-
4132	6	152	4.9358	0.050	0.20	0.40	50
4135	6	154	5.2232	0.041	0.164	0.398	42
4139	6	175	3.6218	0.035	0.140	0.30	47
4140	6	174	1.2610	0.032	0.128	0.255	50
4136	8	235	6.0869	0.027	0.108	0.246	44
4172	8	170	3.9194	0.047	0.188	0.40	47
4138	8	185	4.7272	0.038	0.152	0.266	57
Average				0.0381	0.152	0.330	48.5

conversion hysterias had a tendency to hyperventilation. He made his observations from spirometric tracings of 35 patients in whom a diagnosis of neurosis had been made. Clinically there was no evidence of organic disease in these cases.

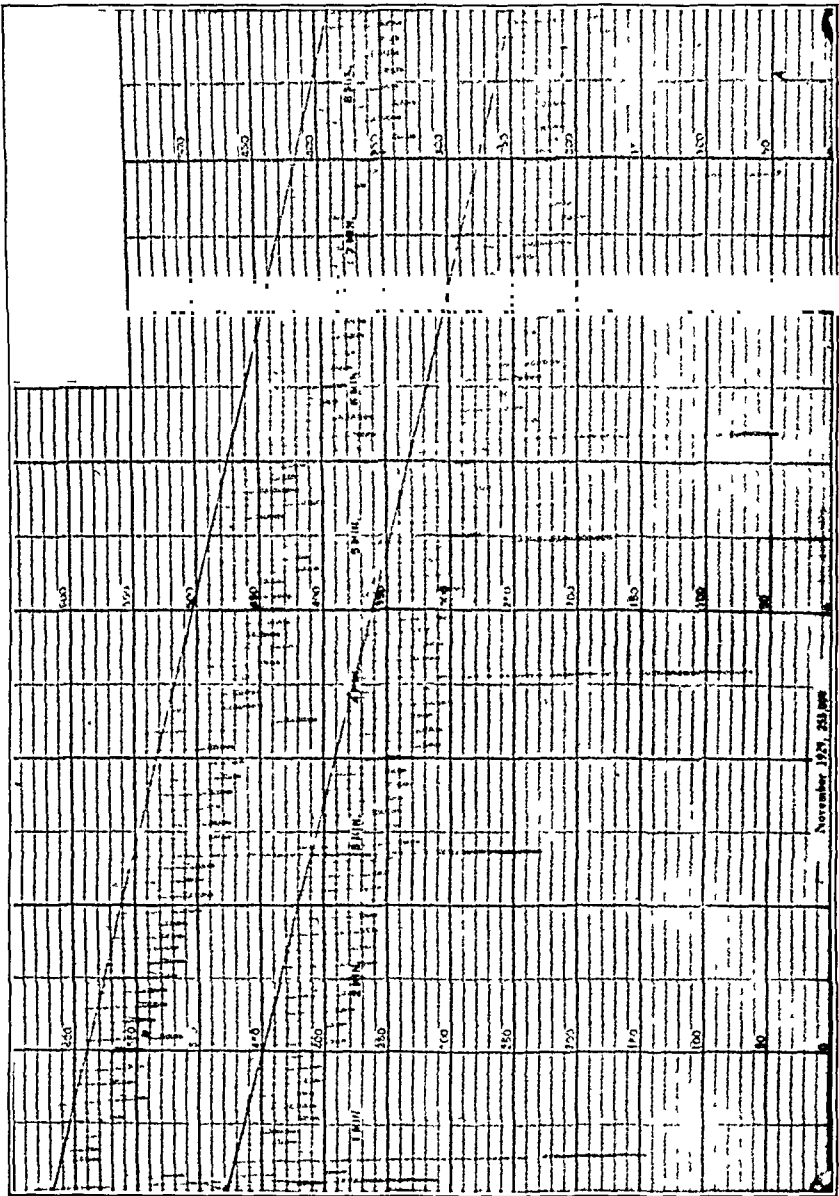


Chart 1.

CASE 1.—Man, aged thirty-five years, complained of nervousness, periodical quivering of muscles, insomnia, and restlessness. Two months before, he had experienced dyspnea on exertion, precordial distress, and a tachycardia with rate of 200. Four years earlier he had dreaded going to the hospital where he had had a bilateral inguinal herniotomy, and since then

rachitic groups. With the exception of the normal group killed at the age of six weeks and three days, the absorption and storage of iron, as indicated by the total liver iron, was increased in both normal and rachitic groups. However, there was no significant variation in the total iron content of the livers, after seven and fourteen days of iron supplement as indicated by the figures for the normal and rachitic chicks killed at the age of seven and eight weeks. These results, obtained by the experimental methods described, suggest that there is no interference with the absorption and storage of iron in the rachitic chick.

#### DISCUSSION

Although these experiments indicate that anemia does not occur as a symptom of rickets in the chick, the question arises as to whether or not the rachitic picture in the chick may be compared with that of the human subject. Studies of the blood of chicks rendered rachitic on the rations used in these experiments reveals alterations in the blood chemistry similar to those encountered in infantile rickets. These changes consist of a reduction of serum calcium and phosphorus. Studies of the bone reveal a reduction in the calcium and phosphorus of the bone when rickets is produced on these rations. However, the development of severe rickets in the chick is very rapid and becomes quite marked in the third and fourth weeks of life. When we consider the brief life span of the chick, as compared to that of the human subject, it is conceivable that the period of the development of infantile rickets, usually during the first six months to one year of life, may well be compared to the period of development of rickets in the chick. There are, of course, many unknown factors which may play a part in the production of rickets in the child, while in the experimental work with the chick, we were dealing with a true vitamin D deficiency.

Maughan<sup>10</sup> has recently reported the development of hemoglobinemia in the young rachitic chick. Although no information as to the type of diet used in his studies is given, severe rickets was produced in seven weeks. Hemoglobin readings, as determined by the Hellige solid plane hemometer at the age of seven weeks, were from 2 to 4 gm. below the level of the normal controls. Irradiation of the rachitic chicks without other therapy produced a prompt rise in the blood hemoglobin to normal levels. By the methods used in our experiments, we have been unable to demonstrate such a reduction in hemoglobin.

Shelling and Josephs,<sup>11</sup> in 1934, performed some interesting experiments with normal rats. They found that rats fed a diet high in calcium and low in phosphorus had a lower percentage of hemoglobin in the blood than did those fed on a normal diet. They showed that iron retention, as measured by the total iron content of the desiccated animal, was much lower in rats fed high calcium levels. They also demonstrated that iron retention was increased by the administration of viosterol in rats fed high phosphorus levels but was not increased in animals fed high calcium. Although no conclusions were drawn from these experiments they suggest a definite relationship between disturbances of calcium and phosphorus metabolism and anemia. The theory was advanced that the iron was carried down in the intestinal tract either before absorption had taken place or during the process of excretion.

he felt at times that he were dying. The laboratory examinations of urine, the blood, and Wassermann reaction, as well as complete gastrointestinal x-ray study, and basal metabolic rate of -3 per cent, were normal.

CASE 2.—A stenographer, divorced, aged thirty-two years, complained of nervousness and depression. She had periods when she felt she would suffocate and times when she was afraid she would commit suicide. She had been nervous since the birth of a child thirteen years ago and noted a sensation of abdominal pressure since an appendectomy seven years ago. On examination, indefinite tenderness of the right lower abdomen and a hypertrophic endocervicitis were found. The urine and blood were normal. The Wassermann was negative. The basal metabolic rate was +10 per cent.

CASE 3.—Woman, aged twenty-seven, single, complained of nervousness, insomnia, a choking sensation and feeling of enlargement of the neck for five years. She had outbursts of weeping, and for the last year there had been palpitation of the heart after excitement or exertion. Examination revealed an intelligent woman who was nervous, without exophthalmus and with a thyroid gland whose right lobe was hard, nodular, and four times normal size. The heart was found to be normal. Her hands were cold and moist. The laboratory reported a negative blood Wassermann, a normal blood count and urine. The basal metabolic rate was +22 per cent on June 16, 1933. On July 19, 1933, the patient had a thyroidectomy and the pathologic diagnosis was cystic adenoma. The patient returned on Aug. 7, 1933, complaining of regurgitation immediately after eating. A gastrointestinal x-ray study revealed no abnormalities of the esophagus, stomach, or duodenum. The appendix, however, was found to be tender, and after forty-eight hours was visualized by the roentgenologist, who considered it to be diseased. The basal metabolic rate checked on Oct. 30, 1933, was +10 per cent, and shortly afterward an appendectomy was performed. Two years later the patient was still regurgitating her food and complaining of palpitation when nervous.

A review of these tracings demonstrates a respiratory pattern of irregular breathing and deep sighs. The usual spirometer tracing does not have these irregularities. It is of particular interest to follow the two graphs of Case 3. The first tracing shows evident lack of balance of the respiratory mechanism and reflects the high degree of anxiety due to the patient's emotional reaction to her moderate physical disability. The second tracing, taken three months later, still shows sighing respiration, but as indicated by her subsequent improvement, this is less extreme than it was.

It is profitable to inspect the actual tracing rather than accept only the calculated rate of metabolism. A bizarre pattern suggests the possibility of an environmental or psychogenic component in the patient's reaction. The evaluation of this too frequently neglected factor will in turn give a more exact insight into the patient's disease.

#### REFERENCES

1. Nielsen, J. M., and Roth, P.: Clinical Spirography; Spirograms and Their Significance, Arch. Int. Med. 43: 132, 1929.
2. Ziegler, L. M., and Levine, B. S.: Influence of Emotional Reactions on Basal Metabolism, Am. J. M. Sc. 169: 68, 1925.
3. Baker, D. M.: Sighing Respiration as Symptom, Lancet 1: 174, 1934.
4. Craig, H. R., and White, P. D.: Etiology and Symptoms of Neurocirculatory Asthenia; Analysis of 100 Cases With Comments on Prognosis and Treatment, Arch. Int. Med. 53: 633, 1934.
5. Christie, R. V.: Some Types of Respiration in Neuroses, Quart. J. Med. 4: 427, 1935.

# LABORATORY METHODS

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## THE VALUE OF THE BLOOD XANTHOPROTEIN REACTION IN DIAGNOSIS AND PROGNOSIS\*

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### INTRODUCTION

IN THE search for a simple and reliable test which would enable the clinician to determine or exclude quickly renal insufficiency as cause of coma, it was suggested to me by Jaffe to try the xanthoprotein reaction which Becher had found to be highly satisfactory.

The rôle of the phenols in the xanthoprotein reaction was first pointed out by Salkowski. Moirer found that it was positive with tyrosine and tryptophane. Rakestraw (1923) reported from 17 to 32 mg phenols per cubic centimeter in the blood of normal individuals. Theis and Benedict (1918) found from 1.9 to 8 mg of phenols in 100 cc of blood in a variety of pathologic conditions, while Becher, Litzner, and Taghieh (1925 and 1926) give the normal phenol content of the blood as 0.04 to 0.12 mg per 100 cc.

The aromatic substances of the blood are derived from the oxidation of tryptophane, tyrosine, and phenyl alanine in the body metabolism or in putrefaction in the intestine. Under pathologic conditions there is a definite increase of these substances in the blood. Renal insufficiency, liver damage with changes in the deamidization function and increased protein destruction, metabolic disorders and neoplasm with a large amount of tissue destruction will increase the amount of phenols in the blood.

The study of the aromatic substances by the xanthoprotein reaction in the blood in normal and pathologic individuals has been carried out very thoroughly by Becher with his associates. In 1924 he reported an increase of the aromatic substances, an ether soluble group comprising phenols, cresols, aromatic oxyacids, and indoxyl, and another nonether soluble group containing the amino acids, tyrosine-phenyl alanine and tryptophane occurring in renal insufficiency. Other conditions, liver damage, cardiac decompensation, infections, ileus, pernicious anemia, and cadaver blood gave an increased xanthoprotein reaction.

The intensity of the reaction was measured by comparing it with a standard aqueous solution of 0.03874 per cent potassium dichromate in an Autenrieth colorimeter.

More accurate methods were devised to determine quantitatively the amount of phenol present. Becher, Litzner, and Taghieh (1926) reported a

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TABLE I

BLOOD STUDIES AND X-RAY FINDINGS OF 30 INFANTS AND CHILDREN SUFFERING FROM UNCOMPLICATED AND COMPLICATED RICKETS

AGE	HB* %	R.B.C.	X-RAY FINDINGS	CA	P	REMARKS
<i>Uncomplicated Rickets (Active)</i>						
18 mo.	73	5,000,000	Severe	-	-	
16 mo.	45	4,000,000	Moderate	9.4	3.1	
21 mo.	85	6,240,000	Severe	7.8	5.68	
18 mo.	74	4,990,000	Severe	13.7	2.6	
11 mo.	55	4,270,000	Healing	-	-	
12 mo.	55	4,640,000	Severe	7.8	-	
6 mo.	72	3,800,000	Moderate	-	-	
22 mo.	69	4,000,000	Severe fracture	-	-	
10 mo.	52	4,650,000	Severe	9.5	7.0	
30 mo.	94	4,900,000	Moderate	12.7	4.63	
25 mo.	85	5,420,000	Moderate	-	-	
24 mo.	50	4,300,000	Moderate	-	-	
24 mo.	75	4,630,000	Moderate	12.3	3.87	
36 mo.	65	4,800,000	Severe	7.18	4.00	
24 mo.	78	4,500,000	Severe	9.31	3.55	
19 mo.	75	4,800,000	Moderate	10.6	3.8	
19 mo.	65	4,300,000	Moderate	-	-	
11 mo.	65	4,410,000	Mild	11.7	5.5	
11 mo.	50	4,160,000	Severe	9.3	2.9	
<i>Complicated Rickets (Active)</i>						
7½ mo.	55	4,100,000	Early mild	-	-	Poor diet
7½ mo.	59	3,630,000	Mild	-	-	Cleft palate, diet poor
8 mo.	60	4,670,000	Severe	-	-	Spasmophilia
8 mo.	42	3,810,000	Severe	-	-	Upper respiratory infection and otitis media
12 mo.	18	3,880,000	Severe	-	-	Poor diet. Nutritional anemia
17 mo.	35	5,000,000	Moderate	-	-	Poor diet
12 mo.	55	4,900,000	Healing	-	-	Acute suppurative cervical adenitis
12 mo.	70	4,100,000	Mild	-	-	Cleft palate
9 mo.	50	4,600,000	Severe	7.74	-	Spasmophilia-upper resp. infection and otitis media
18 mo.	65	4,410,000	Moderate	5.59	3.4	Spasmophilia and upper resp. infection
30 mo.	70	4,100,000	Moderate	-	-	Cretinism

\*Hemoglobin readings by Tallquist Method.

One group consisting of 15 one-day-old chicks was placed on a basal ration recommended by Hart, Kline and Keenan<sup>6</sup> which consists of:

Yellow corn	59 parts
Wheat middlings	25 parts
Crude casein	12 parts
Ca carbonate	1 part
Ca phosphate	1 part
Dried yeast (A. B.)	1 part
Sodium chloride	1 part

Chicks fed on this ration developed severe rickets in three to four weeks. The control group of 15 one-day-old chicks was placed on the same ration supplemented with 1 per cent of cod liver oil. Hemoglobin determinations, using a modification of the Newcomber method recommended by Schultze and Elvehjem,<sup>7</sup> were made at weekly intervals beginning in the second week. The average results in grams of hemoglobin per 100 c.c. of blood are shown in Table II. It will be noted that the greatest difference in hemoglobin occurred in the fifth week, at

2 Add 5 cc of 20 per cent aqueous solution of trichloroacetic acid to 5 cc of the plasma, serum, or body fluid. Mix thoroughly and allow to stand for ten minutes. Filter.

3 Add 0.5 cc of concentrated nitric acid to 2 cc of clear filtrate obtained from Procedure 2.

4 Heat to boiling for one half minute. Note. In cases that are markedly positive, a yellowish color is obtained at this point in the procedure.

5 Allow to cool and then add 1 cc of a 35 per cent aqueous solution of sodium hydroxide.

6 The reaction is best examined in direct daylight. A positive test gives a golden yellow color.

Investigators have compared the xanthoprotein reaction with standards to determine different intensities of the test. Becher (1924) used a 0.03874 per cent aqueous solution of potassium dichromate in an Autenrieth colorimeter.

Different dilutions of the potassium dichromate solution were placed in Wassermann tubes. The undiluted solution was called 1, a 90 per cent solution was called 2, and so on to a 10 per cent solution which was labeled 10. The xanthoprotein reaction to be graded was placed in a similar Wassermann tube and compared with the different dilutions of the standard.

Many of the xanthoprotein reactions gave a color of a much greater intensity than the Standard 1. This is the usual case in uremia and marked renal insufficiency.

*Observations*—The data for this study came from patients in the wards of the Cook County Hospital or from deceased patients examined in the Department of Pathology of the same hospital. A total of 210 cases were studied and were classified as follows:

	TOTAL CASES
1 Cases admitted in coma	26
2 Cases with xanthoprotein reaction of 1 or stronger	11
3 Cases giving a xanthoprotein reaction from 2 to 10	68
They were further subdivided on the basis of survival and the urea nitrogen findings	
A High urea nitrogen and died	8
B Low urea nitrogen and died	16
C High urea nitrogen and survived	5
D Low urea nitrogen and survived	29
Total cases	68
4 Cases excluding chronic nephritis and coma without blood chemistry determinations	57
5 Cases from which blood was obtained at autopsy	25
6 Cases from which blood and other body fluids were obtained at the postmortem examination	10
Total cases	210

*Coma Cases*—Twenty six cases of patients admitted in coma or stuporous states are listed in Table I. In a few cases indican determinations were made. Four patients gave positive reactions to the xanthoprotein test. Blood chemistry was determined in three of the patients, and in all these patients there was a marked elevation of urea nitrogen. The indican test, although obtained in all four cases, was elevated to a uremic level in only two cases (9 and 18). All the patients died in one to ten days with uremia.

and that portion used for the determination of total iron dried in a 100° oven for twenty-four hours. The available iron was determined by the bipyridine method of Kohler, Elvehjem and Hart.<sup>8</sup> The total iron was determined by the thiocyanate method, or the bipyridine method for total iron recommended by Lintzel.<sup>9</sup> The results for the normal and rachitic groups are recorded in Table III.

From the results obtained, by the methods used in this experiment, it is apparent that we have been unable to show a reduction of the blood hemoglobin in the rachitic chick, or demonstrate a significant difference in the total and available iron content of the normal and rachitic chick livers.

In order to demonstrate, if possible, a relationship between the absorption and storage of iron and the disturbance of calcium and phosphorus metabolism in the rachitic chick, the following experiment was performed. One group of twenty-one-day-old white leghorn chicks was placed on the rachitogenic ration described above. Another group of twenty-one-day-old chicks was placed on this basal ration supplemented with 1 per cent of cod liver oil. Hemoglobin readings were made in the sixth week and average readings for the twenty chicks in each group were 9.59 gm. per 100 c.c. for the normal controls and 8.82 gm. per 100 c.c. for the rachitic chicks. At the age of six weeks, when a severe degree of rickets had developed in the chicks on the basal ration, five chicks from each group were killed and the livers analyzed for their total and available iron content. The remaining chicks of both groups were then given the basal rations supplemented with 0.1 per cent of iron in the form of ferric sulphate. At the end of three days, seven days, and fourteen days, five chicks from each group were killed and the livers analyzed for their total iron content. The results of this experiment are recorded in Table IV. The available iron was not done on

TABLE IV  
THE EFFECT ON THE IRON CONTENT OF RACHITIC AND NORMAL CHICK  
LIVERS PRODUCED BY FEEDING IRON

AGE WHEN KILLED	GROUPS	AVERAGE HB IN GRAMS/100 C.C. (20 CHICKS)	AVERAGE TOTAL IRON (LIVER) MG./GM. DRY LIVER	AVERAGE AVAILABLE IRON (LIVER)	
				MG./GM. FRESH LIVER	MG./GM. DRY LIVER
Six weeks No iron	Normal	9.59	0.332	0.03	0.12
	Rachitic	8.82	0.334	0.038	0.152
Six weeks and 3 days 0.1% added iron for 3 days	Normal		0.30		
	Rachitic		0.42		
Seven weeks 0.1% added iron for 7 days	Normal		0.36		
	Rachitic		0.391		
Eight weeks 0.1% added iron for 14 days	Normal		0.47		
	Rachitic		0.498		

the groups killed after the age of six weeks, because it was felt that the total iron content of the livers gave us enough information about the absorption and storage of the added iron. It will be noted from a study of Table IV that the average total and available liver iron varied very little in the normal and

TABLE I—CONT'D

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL CONDITION	BLOOD				RESULT	FINAL DIAGNOSIS
			XANTH	INDICAN MG %	LI.FA N. MG. %	CREATININE MG %		
19. W-59-M	Uremia and nephrosclerosis	Coma	9	0.15	35	12.3	Died	Hemiplegia encephalomalacia
20. C-31-M	Epileptiform seizures	Coma	6	-	-	-	Released	Epilepsy
21. W-15-M	Subacute bacterial endocarditis	Coma	7	0.05	16	-	Died	Cerebral hemorrhage
22. W-55-M	Acute alcoholism	Coma	7	0.45	-	-	Discharged	Alcoholic coma
23. W-50-M	Malignant endocarditis	Coma	5	-	25	2.0	Died	Postmortem malignant endocarditis
24. C-55-M	Bronchopneumonia	Semistuporous	6	-	16	-	Discharged	Bronchopneumonia
25. W-44-M	Epilepsy	Convulsions	9	-	-	-	Discharged	Epilepsy, traumatic
26. W-21-M	Alcoholism	Irrational	8	-	-	-	Died	Acute alcoholism

Of the remaining 22 patients, 7 recovered and were discharged. The xanthoprotein reactions were low. The other 17 patients died, none of renal insufficiency, although upon admission 7 were diagnosed as such. The urea nitrogen was elevated in 8 patients. The xanthoprotein reaction ranged from 3 to 10.

The results confirm the findings of other investigators. All patients who presented a xanthoprotein value of 1 died of renal insufficiency. No definite conclusions can be established as to the diagnosis or prognosis in patients with lower xanthoprotein values.

The findings, which agree with those reported by Beecher (1930), show that the xanthoprotein reaction was more sensitive than the indican test. In only 2 of the 4 patients with renal insufficiency did the indican content of the blood approach the uremic level.

Unfortunately permission for postmortem examination could be obtained in only 5 patients.

*Cases With High Xanthoprotein Values.*—Table II lists 11 patients with renal insufficiency who survived from one to ten days after the xanthoprotein reaction was obtained, and all died in uremia. The urea nitrogen was determined in 10 cases and ranged from 42.49 to 206 mg. per cent. The xanthoprotein test gave values of one in all cases.

The author concludes from these findings that a patient with a xanthoprotein reaction of one presents a case of marked renal insufficiency. The longest period of survival in the coma group and this series of cases was ten days.

It is entirely possible that a high calcium intake or a high calcium-phosphorus ratio may retard iron assimilation, and we are now extending our studies to this question. However, rickets in infants is most likely due to low calcium or vitamin D intake rather than a disturbed calcium-phosphorus balance. Repeatedly we have seen anemia in rickets fail to respond to the administration of cod liver oil or viosterol without additional measures. The administration of iron salts to rachitic infants, without supplementing the diet with cod liver oil, raises the hemoglobin levels satisfactorily.

From the clinical evidence and the experimental results with the chick, it may be concluded that anemia is not a symptom of rickets but is probably secondary, in the infant, to complicating factors such as nutritional deficiency or infections. In very advanced, severe rickets, the poor appetite for supplementary foods and lowered resistance to infection are probably the greatest factors in the production of anemia.

#### SUMMARY

1. The problem of anemia in rickets is briefly reviewed from a clinical viewpoint.

2. We have been unable to demonstrate a significant difference in the hemoglobin content of the blood of normal and rachitic chicks. The hemoglobin readings were made between the ages of two to six weeks.

3. Analyses of the livers of normal and rachitic chicks revealed no significant difference in the total and available iron content.

4. In the rachitic chick, the abnormal calcium and phosphorus metabolism had no effect on the absorption and storage of iron so far as could be demonstrated by the methods used in our experiments.

The authors wish to express their appreciation for the suggestions and encouragement of Dr. C. A. Elvehjem of the Department of Agricultural Chemistry and Dr. J. E. Gonce, Jr., of the Department of Pediatrics.

#### REFERENCES

1. Hess, A. B.: *Rickets, Including Osteomalacia and Tetany*, Philadelphia, 1929, Lea and Febiger, p. 245.
2. Findlay, L.: *The Blood in Rickets*, *Lancet* 1: 1164, 1909.
3. Griffith, J. P. C., and Mitchell, A. G.: *The Diseases of Infants and Children*, Philadelphia and London, 1933, W. B. Saunders and Co., p. 451.
4. Royster, L. T.: *Clinical Pediatrics* 10: New York and London, 1927, D. Appleton-Century Co., p. 241.
5. Hess, A. F.: *Abt's Pediatrics* 2: Philadelphia and London, 1923, W. B. Saunders and Co., p. 936.
6. Hart, E. B., Kline, O. L., and Keenan, J. A.: *A Ration for the Production of Rickets in Chicks*, *Science* 73: 710, 1931.
7. Schultze, M. O., Elvehjem, C. A., and Hart, E. B.: *An Improved Method for the Determination of Hemoglobin in Chicken Blood*, *J. Biol. Chem.* 105: 253, 1934.
8. Kohler, G. O., Elvehjem, C. A., and Hart, E. B.: *Modification of the Bipyridine Method for Available Iron*, *J. Biol. Chem.* 113: 49, 1936.
9. Lintzel, Wolfgang: *Zur Methodik der Mikrobestimmung des Eisens in Biologischem Material*, *Ztschr. ges. Exper. Med.* 86: 269, 1933.
10. Maughan, G. H.: *Hemoglobin Studies in Rachitic Chickens; the Effect of Ultra-Violet Irradiation*, *Proc. Soc. Exper. Biol. & Med.* 32: 389, 1934.
11. Shelling, D. H., and Josephs, Hugh: *Calcium and Phosphorus Studies: X, The Effects of Variation of Calcium, Phosphorus and Vitamin D in Diet on Iron Retention in Rats*, *Bull. Johns Hopkins Hosp.* 55: 309, 1934.

TABLE III  
LOW XANTHOPROTEIN REACTION—HIGH UREA NITROGEN, PATIENTS DIED

CASE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD				DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N MG %	CREATININE MG %	XANTH	INDICAN MG %		
1. C-64-M	Uremia in coma	Coma	100	9.5	9	0.15	5	Coronary throm- bosis
2. C-105-M	Prostatic hyper- trophy	Senile	55	4.0	8	2.0	23	Prostatic hyper- trophy; alkaline cystitis
3. W-62-F	Hypertension Uremia	Semistupor- ous	48	2.4	9	0.05	1	Post-mortem—Mod. ecc. hypertrophy of heart
4. C-38-F	Chr. morphinism Asthma		56	3.6	4	1.5	34	Post-mortem—Genl. amyloidosis (chr. morphinism)
5. W-68-M	Syphilitic aortitis	Decom- pensated	11/7/34 11 2.1 1/27/34 43 2.6	3/15/34 8	-	16	Malignant endo- carditis	
6. W-33-M	Chr. glomerulo- nephritis; uremia	Coma	11 4.0	6	-	19	Postmortem—Chr. glomerulonephritis and bronchopneu- monia	
7. C-67-M	Hypertension with arterio- sclerosis		11/6/33 115 10.0	11/4/33 4	1.0	23	Arteriosclerosis	
8. C-32-F	Chr. glomerulo- nephritis		12/6/33 116 1 -	12/12/33 4	2.0	48	Post-mortem—Chr. glomerulonephritis	

Three of the cases were diagnosed as chronic glomerulonephritis at the postmortem examination. The blood chemistry and the xanthoprotein reactions were performed some time before death. A check on the xanthoprotein reaction at a later date would have been very desirable.

In two patients listed in Table III and in 9 patients listed in Table IV, the xanthoprotein reaction was performed shortly before death. The patients died of conditions other than renal involvements, and all had low xanthoprotein values. In the other cases too much time elapsed between the test and death to make conclusions of any value.

Table V lists 5 cases of patients, all with elevated blood urea nitrogen, who either left the hospital by discharge or release.

The 39 cases listed in Table VI present a wide range of diagnoses. The blood nitrogenous substances were not increased; the xanthoprotein reaction ranged from a value of 4 to 10, most of the values being in the group between 5 and 10. All the patients left the hospital improved.

Of interest is one case, Case 30, diagnosed as acute glomerulonephritis with a low xanthoprotein value of 8. This finding confirms Becher (1925) and other investigators who reported little or no increase of aromatic substances in acute nephritis.

colorimetric procedure, utilizing the Millon reaction and a comparing solution of phenol cresol. They determined free and bound phenols and found values up to 3.5 mg. per cent in different pathologic conditions. Kammer (1933) determined the phenol content by using a tyrosine solution as a standard in a colorimeter and found values up to 5.55 mg. per cent.

However, Becher (1924) pointed out that it was not necessary to gauge the strength of the reaction to determine the presence of renal insufficiency. A definitely positive reaction gave a strong golden color which was unmistakable and could be easily recognized.

Becher and Koch (1925) pointed out that uremia was less dependent on the retention of nitrogenous products, that phenol poisoning resembled true uremia and that the aromatic substances played an important rôle in the pathogenesis of uremia.

Becher (1925) found a slight or no increase of the phenols in the blood of patients with acute nephritis, although there may be a considerable increase in the urea and uric acid. Granular atrophy of the kidney produced a definite increase of the phenols, while true uremia presented the highest increase.

The value of the test for prognosis and as an aid in differential diagnosis especially in comatose conditions was emphasized by Boeminghaus (1926), Tonietti (1929), Scherk (1927), Hoesch (1931), Zamyslowa (1931), and others.

A large literature has evolved from the work of Kammer, Widenhorn, Gromer, Goldman and Burniewicz, Inoue, Rathery and Waitz, Tschilow, Melly, and Wuhrman, who have found high values of the xanthoprotein reaction in uremia, chronic nephritis, arteriosclerotic kidney, with elevated values in creosote poisoning, enteritis, volvulus of intestines, thyrotoxicosis, cardiac decompensation, liver cirrhosis and atrophy, pneumonia, diabetes mellitus, carcinoma, brain tumor, pernicious anemia, lung involvements, blood dyscrasias, agonal states, cadavers, and after large doses of salicylates. Low values were found in acute nephritis.

Of special interest is the question as to what effect phenol poisoning has on the blood xanthoprotein reaction. Smith (1933) reported a study of phenols in rabbits. He found approximately 0.5 mg. per cent of phenols in different tissues of the normal animal. Free phenols, ranging from 7 to 26 mg. per cent, were found in various tissues of animals after the administration of lethal doses of phenol and orthocresol. Becher, Litzner and Taglich (1926) found 1.4 per cent mg. free and 2.1 mg. per cent bound phenol in a case of severe lysol poisoning. Incidentally, this was the highest value obtained in a study of phenols in a variety of conditions. Goldman and Burniewicz (1928) studied a case of creosote poisoning and obtained a blood xanthoprotein reaction of 45 per cent. Such findings make it necessary to include poisonings from various phenol compounds with conditions giving a strong blood xanthoprotein reaction.

#### *Technic of the Xanthoprotein Reaction.—*

The xanthoprotein reaction was performed as described by Becher (1924).

1. Whole blood, plasma, serum, body fluids, i.e., pericardial, pleural or peritoneal, or cerebrospinal fluid may be used. It is wise in the case of whole blood to secure 10 c.c. and centrifuge.

TABLE V  
LOW XANTHOPROTEIN—HIGH UREA NITROGEN—PATIENTS DISCHARGED

FACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD			DISPOSITION DAYS AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA MG %	CLATININE MG %	XANTH		
1 W-47-M	Syphilitic heart disease	Acute illness	36	1.3	6	Discharged 45 days	Coronary throm- bosis Hemiplegia
2 W-54-M	Impending uremia	Convulsion	45	3.8	7	Discharged 15 days	Chronic glomerulo- nephritis
3 W-51-F	Generalized arteriosclerosis	Condition poor	131	3.5	3	Released 1 day	Uremia with chronic nephritis
4 W-61-M	Thyrototoxicosis		5	2.4	7	Released 7 days	Thyrototoxicosis
5 W-32-M	Nephrotic syn- drome		5	5.4	7	Discharged 2 days	Chronic glomerulo- nephritis

ogy in 2 cases, and 1 case each of subacute bacterial endocarditis, cerebral hemorrhage, suppurative leptomenigitis, and carcinoma of the pancreas.

The xanthoprotein reactions obtained from the 2 cases of liver cirrhosis and the 1 case of carcinoma of the pancreas with liver metastases agree with the findings of other investigators. Tschlow (1929) reported high values in different types of liver diseases. Hoesch (1931) found elevated xanthoprotein values in cases of liver cirrhosis, acute liver atrophy, liver and bile duct carcinoma, and severe cholangitis. He believed that finding of a high xanthoprotein reaction, a relatively low nonprotein nitrogen and urea, along with a negative indican, could be used diagnostically to rule out renal disease, especially in comatose conditions.

The anatomic diagnosis showed renal pathology in 7 other cases, with xanthoprotein reactions ranging from 2 to 9.

The remaining 14 cases showed xanthoprotein values from 3 to 10.

Wuhrman (1935) studied the nonprotein nitrogen and the xanthoprotein reaction in living, agonal, and postmortem states and found an increase of these values in the agonal and postmortem states. This work casts doubt on the validity of results, such as the ones obtained in this series of postmortem cases.

*Xanthoprotein Reaction Compared in the Blood and Body Fluids*—In 13 postmortem cases listed in Table VIII, the xanthoprotein reaction was performed on the heart blood, and on the pericardial fluid, on 2 cases of peritoneal fluid, and on two cases of pleural fluid. Chemical determinations were obtained on the pericardial fluid of 7 cases.

It is interesting to note that in 6 cases the same values for the xanthoprotein reaction were obtained in the blood and pericardial fluid, while in Case 5 the same value was obtained in the blood, peritoneal, and pericardial fluids. In some of the other cases there was a close agreement between the different values. Beecher (1924) likewise found a corresponding increase in the xanthoprotein reaction of blood and body fluids.

TABLE I  
COMA CASES

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL CONDITION	BLOOD				RESULT	FINAL DIAGNOSIS
			XANTH.	INDICAN MG. %	UREA N. MG. %	CREATININE MG. %		
1. C-70-M	Hypertensive heart disease	Irrational Stuporous	6	0.25	37	2.3	Died	Hypertensive heart disease
2. W-76-M	Organic heart disease and exposure	Semicomatose	6	-	15	-	Died	Cerebral accident
3. C-65-M	Uremia	Coma	10	0.05	18	-	Died	Postmortem ecc. hypt. of heart
4. C-50-M	Chr. nephritis with uremia	Coma	8	-	8	-	Died	Postmortem mod. eccentric hypertrophy of heart
5. W-61-M	Syphilitic thrombosis	Stuporous Aphasic	7	-	15	-	Discharged	Cerebral hemorrhage
6. W-7-M	Hypertension with cerebral accident	Coma	7	-	15	-	Died	Cerebral hemorrhage
7. W-66-M	Hypertension and nephrosclerosis	Irrational Unable to speak	5	-	24	1.8	Died	Syphilitic cerebral thrombosis
8. C-37-F	Uremia, Vincent's angina	Coma	5	1.0	80	6.6	Died	Acute tonsillitis also marked dehydration
9. W-56-M	Uremia	Semistuporous	1	4.0	160	10.0	Died	Uremia, lived 1 day
10. C-55-F	G. I. Malignancy, uremia	Responded very poorly	1	1.5	73	6.6	Died	Chr. nephritis Uremia, lived 10 days
11. C-54-F	Hypertensive heart disease	Coma	1	2.0	126	19.0	Died	Postmortem Ca. of cervix with obstruction of ureters with uremia. Lived 3 days
12. C-60-M	Diabetic coma	Semiconscious and irrational	3	-	29	2.0	Died	Diabetic coma
13. W-68-M	Generalized arteriosclerosis	Irrational	7	-	18	-	Discharged	Coronary sclerosis
14. W-35-M	Alcoholic coma	Coma	7	-	13	-	Discharged	Alcoholism with coma
15. C-64-M	Uremia	Coma	9	0.15	100	9.5	Died	Coronary thrombosis
16. C-105-M	Hypertrophied prostate	Senile	8	2.00	55	4.0	Died	Prostatic hypertrophy and alkaline cystitis
17. W-62-F	Hypertension uremia	Semistuporous	9	0.05	48	2.8	Died	Postmortem mod. ecc. hypt. of heart
18. C-53-M	Carcinoma of prostate	Disoriented	1	3.0	-	-	Died	Ca. of prostate and arteriosclerosis with uremia. Lived 4 days

TABLE VII  
POSTMORTEM CASES

POSTMORTEM NO	FACT AGE SEX	PREMORTEM BLOOD CHEMISTRY		POSTMORTEM BLOOD		HOURS DEAD	POSTMORTEM DIAGNOSIS AND COMMENTS
		UREA N MC %	CHLORINE MC %	XANTH	INURAN MC %		
1 1017	C 21-M			10	(0)	17	Ruptured aortic aneurysm
2 1052	W 46-F				10	4	Chronic glomerulonephritis Coma before death
3 1122	C 66-F	2	17			2	Syphilitic aortitis
4 1149	W 23-F				0.0	4	Ruptured aneurysm of cerebral artery
5 1155	C 20 F				0.1	11	Generalized sepsis Acute diffuse interstitial nephritis
6 4	W 59 M				1.0	14	Carcinoma of prostate Malignant endocarditis
7 19	W 42 M	80	7.0		0		Stricture of urethra Bilateral pyelonephrosis and ascending suppurating pyelonephritis
8 34	W 72 M	2	2.0		0.0	1	Carcinoma of prostate
9 37	W 34-F				0.0		Cystic supracellar adenoma tumor
10 64	C 41-F		17			44	Fibrous pulmonary tuberculosis
11 65	W 58-F						Sepsis lenta
12 95	W 48 M	168		1	1.0	71	Malignant nephrosclerosis
13 244	W 50 M	102	7.0	1	1.0	34	Chronic glomerulonephritis
14 317	W 47-M	41	2.6	4	0.2	9	Subacute to chronic glomerulonephritis
15 386	W 29 M			1	1.4		Subacute vegetative endo- carditis
16 411	W 63-M	66	4.0	1	2.0	14	Renal decompensation of be- nign nephrosclerosis and confluent bronchopneumonia
17 428	W 57-M	9		1		24	Periportal cirrhosis of liver
18 492	C 40 F	100	9.0	1	2.5	19	Fibrinous pericarditis and cyst of left ovary with compression of the uterus and left ureter
19 496	W 48-F	15		5	0.05	2	Hydropicardium Hyper- trophy of left ventricle of heart Arterio sclerotic granulations of kidneys
20 500	W 38-M			1		24	Cerebral hemorrhage
21 508	C 38 M	62	4.4	6		154	Ancient myomata of apex of heart
22 516	W 54-M			1		54	Suppurative leptomeningitis (otitis)
23 528	C 54-F	126	19.0	1			Ulcerated carcinoma of the cervix and compression of the uterine openings
24 530	C 38-F	44	2.5	1	0.5	16	Malignant nephrosclerosis Xanth 56 days before death
25 548	W 86-M			4		14	Recent encephalomalacia of left side of pons
26 552	C 50 F			1		2	Carcinoma of the pancreas
27 559	W 39 M	12		1		9	Periportal cirrhosis of the liver
28 569	C 34-F			5	1.0		Ulcerative esophagitis with perforation and suppurative lymphadenitis of posterior mediastinum

TABLE II  
HIGH XANTHOPROTEIN REACTIONS—PATIENTS DIED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD				DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	CREATININE MG %	XANTH. MG %	INDICAN MG %		
1. C-60-M	Carcinoma of prostate	Developed pericardial friction rub	106	8.0	1	2.0	5	Postmortem—Adenocarcinoma of prostate with obstruction of urinary bladder and uremia
2. W-47-M	Chronic nephritis	Developed comatose state	105	8.5	1	1.5	½	Chronic nephritis with uremia
3. C-46-F	Arterio-sclerosis; hypertension		206	8.4	1	3.0	1	Postmortem—Malignant nephrosclerosis with uremia
4. W-43-M	Asthmatic bronchitis, nephritis, pre-uremia	Developed convulsive state. Spinal Wass. 4+	86	10.0	1	1.0	5	Syphilitic meningitis; uremia
5. C-32-F	Chronic nephritis with hypertension		65	4.2	1	7.1	2	Postmortem—Malignant nephrosclerosis. Fibrinous pericarditis with uremia
6. C-45-M	Hypertensive heart disease	Decompensated	191	14.0	1	3.0	6	Postmortem—Ecc. hypertrophy of heart. Arterio-sclerotic kidneys with uremia
7. C-35-M	Hypertensive heart disease	Decompensated. Pericardial friction rub	42	2.4	1	2.9	4	Postmortem—Chronic nephritis with uremia
8. W-56-M	Uremia, hemiplegia	Semistuporous	160	10.0	1	4.0	1	Uremia
9. C-55-F	Gastrointestinal malignancy; uremia	Stuporous	73	6.6	1	1.5	10	Chronic nephritis with uremia
10. C-54-F	Hypertension and hypertensive heart disease	Coma	126	19.0	1	2.0	3	Postmortem—Ca. of cervix with obstruction to the ureters with uremia
11. C-53-M	Carcinoma of prostate	Disoriented	-	-	1	3.0	4	Ca. of prostate. Arteriosclerosis with uremia

*Low Xanthoprotein Reactions.*—This group comprises 68 cases in which the xanthoprotein reaction was below the value of one, considered low, or negative. The results were not uniform; the diagnoses were varied, and the prognoses were uncertain.

Tables III and IV list the data of 24 patients who died, one group with a high urea nitrogen value and another group with low values. The xanthoprotein values ranged between 3 and 9. Two patients showed an indican value of 2.0 mg. per cent just below the uremic level.

uremia All the cases in this series with a xanthoprotein reaction of one or stronger were diagnosed as cases of renal insufficiency, and died For practical purposes any xanthoprotein reaction of a dark yellow, golden color may be considered positive or equivalent to the values expressed as one in this work According to the work of Rose and Alving the xanthoprotein reaction with a value of one is equivalent to a tyrosine content of the blood of 7.2 mg per cent and a kidney function of 10 per cent of normal Many of the xanthoprotein reactions with a positive value are more intense than the 0.03874 per cent potassium dichromate solution Hence, a phenol concentration equivalent to a tyrosine concentration of 7.2 mg per cent or higher must be considered when evaluating the xanthoprotein reaction of one

The author concludes that the real significance of the xanthoprotein reaction is in the information obtained from a positive value of one or stronger

Xanthoprotein reactions of less intensity than one were found hard to evaluate Values from 2 to 5 were found in a great variety of patients, some of whom lived, and some of whom died However, in general, the patients with values between 5 and 10 improved lived and were discharged

As has been pointed out by other investigators, no correlation was found between the aromatic and nitrogenous products in the blood

The findings—that, in general the indican and the xanthoprotein reactions parallel each other, but in some cases the indican may be low and the xanthoprotein high—confirm the results obtained by Becher

The author concludes that the xanthoprotein reaction is more sensitive than the indican test in the diagnosis and prognosis of renal insufficiency

The xanthoprotein reaction of one proved to be 78.5 per cent correct in the diagnosis of marked renal insufficiency and liver disease in the postmortem cases

A general correlation was observed between the xanthoprotein reaction in the blood, the pleural, pericardial, and peritoneal fluids, indicating that a similar increase of aromatic substances was found in the body fluids when they were augmented in the blood

The xanthoprotein reaction commends itself in that it is easily, rapidly, and accurately performed with a definite aid to diagnosis and prognosis

#### REFERENCES

- 1 Becher, E. Über das Vorkommen aromatischer Gruppen in entweisstem Blut, Körperflüssigkeiten und Geweben, nachgewiesen am Ausfall der Xanthoproteinreaktion, München med Wchnschr 71 1677, 1924
- 2 Becher, E. Über eine neue einfache Methode zu Feststellung der Niereninsuffizienz im Blut, München med Wchnschr 71 1611, 1924
- 3 Becher, E. Studien über das Verhalten der Xanthoproteinreaktion im entweissten Blut unter normalen und pathologischen Verhältnissen, Deutsche Arch f Klin Med 148 159, 1925
- 4 Becher, E. Über Unterschiede im Verhalten des Blutes bei Niereninsuffizienz der akuten Nephritis und der Schrumpfnieren und die Verwertbarkeit der Xanthoproteinprobe im einweissfreien Blutfiltrat für die Diagnose und Prognose der Niereninsuffizienz, München med Wchnschr 72 1020, 1925
- 5 Becher, E., and Koch, F. Über die pathogenetischen Beziehungen zwischen echter Uramie und den bei Niereninsuffizienz im Blut retinierten Substanzen, Deutsche Arch f Klin Med 148 78 1925
- 6 Becher, E., Litzner, S., and Tagheli, W. Über das Vorkommen von Phenolen im normalen Blut, über ihren qualitativen und quantitativen Nachweis mit der Millonschen Reaktion und über bemerkenswerte Blutphenolwerte bei Krankheiten insbesondere bei perniziöser Anaemie, München med Wchnschr 72 1676, 1925

TABLE IV

LOW XANTHOPROTEIN REACTION—LOW UREA NITROGEN, PATIENTS DIED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CLINICAL COURSE	BLOOD					DAYS ALIVE AFTER XANTHOPROTEIN TEST	FINAL DIAGNOSIS
			UREA N. MG. %	GELATININE MG. %	XANTH.	INDICAN MG. %			
1. W-59-M	Exposure; malnutrition	Acutely ill	21	-	7	-	13	Coronary sclerosis and hypostatic pneumonia	
2. W-70-M	Arteriosclerosis		18	-	6	-	7	Arteriosclerosis with diabetic gangrene	
3. C-68-M	Arteriosclerosis	Acutely ill	26	1.8	7	0.05	27	Arteriosclerotic heart disease	
4. C-59-M	Pulmonary tuberculosis		10	-	9	-	5	Bronchiectasis and lung abscess	
5. W-50-M	Malignant endocarditis	Acutely ill	28	2.0	5	-	1	Postmortem—Malignant endocarditis	
6. W-68-M	Carbuncle, arteriosclerosis, diabetes		18	-	6	-	51	Carbuncle of shoulder and diabetes	
7. W-63-M	Carcinoma of stomach and coronary sclerosis		23	1.7	7	-	13	Organic heart disease	
8. W-67-M	Prostatic hypertrophy		37	2.3	6	-	4	Prostatic hypertrophy and organic heart disease	
9. W-36-M	Malignant nephrosclerosis		32	2.0	7	0.05	13	Postmortem—Chr. glomerulonephritis	
10. W-69-M	Malignancy or cirrhosis	Acutely ill	-	-	8	-	34	Carcinoma of stomach	
11. W-57-M	Prostatic hypertrophy		16	-	3	0.05	2	Prostatic hypertrophy	
12. C-30-M	Acute rheumatic arthritis		11	-	3	-	85	Postmortem—Psoas abscess, tuberculous caries lumbar vertebrae	
13. C-49-M	Coronary disease	Decompensated	31	2.1	7	-	8	Senility	
14. C-70-M	Aortitis	Decompensated	19	-	9	-	6	Hypertensive heart disease with aortitis. Bronchopneumonia	
15. W-62-M	Carcinoma of thyroid	Acutely ill	20	-	7	-	4	Carcinoma of lung	
16. W-54-M	Hypertensive heart disease	Congestive failure	15	-	8	-	1	Hypertensive heart disease	

*Routine Cases.*—The blood from 57 routine cases of patients, with the exception of comas and nephritics, was obtained at the time of admission for study of the aromatic substances. All the xanthoprotein values were low, ranging from 4 to 10.

*Postmortem Cases.*—The xanthoprotein reaction was performed on the heart blood of 35 cases at postmortem. The test gave a value of 1 in 14 cases. The anatomic diagnosis revealed kidney involvement in 8 cases, liver pathol-

## AN INEXPENSIVE FORBUS TYPE AUTOPSY TABLE\*

GEORGE H. FLITTMAN, M.D. AND SAM T. HIDINGER, MAYVIEW, PA.

RECENTLY it became necessary to replace an outmoded autopsy table at this institution. Because of the exorbitant prices asked by supply houses for tables offering any conveniences beyond those inherent in a diamable stationary slate or metal slab, recourse was had to the literature, with the thought of constructing a table from published plans. After some deliberation, the table designed by Forbus<sup>1</sup> was taken as a model and using salvaged materials for the most part, a table was built by the maintenance department of this institution.

The table, which has now been in use for several months, has been more than satisfactory. In view of its low cost without sacrifice of convenience, we thought it worth while to publish complete plans and specifications of our model. Our plans present no great departures from the general design of the original, but do provide for many substitutions of material by which sizable economies were effected without loss of utility. The changes we have made may be appreciated by comparing our specifications with the specifications contained in Forbus' article. Labor and material costs are given as of 1936. Although practically all of the materials which we utilized were salvaged, costs given here are for new materials. Plans and a photograph are included which convey an adequate impression both of the details of construction and the finished appearance.

### SPECIFICATIONS

*Table Top*—The top consists of seven pieces of light gray marble,  $\frac{7}{8}$  of an inch thick, set with waterproof cement in an angle iron frame  $32\frac{1}{4}$  by  $93\frac{3}{4}$  inches constructed of 2 by 2 angles  $\frac{1}{4}$  of an inch thick. Angle members support all joints throughout their entire lengths, thus making the table top watertight. The working surface is highly polished and is graded one inch from the head of the table to a drain at the foot. The two pieces forming the main part of the table top are fitted tightly inside the end and side curbs which locks the curbs between the main part of the table and the angle iron frame as shown in Sections AA and BB. A shelf 15 inches wide extending across the width of the table is sloped  $\frac{1}{4}$  of an inch from the foot to the drain. The edge of the shelf at the drain is bull nosed (see Section AA).

Twenty-six inches from the inside of the head and on the left side of the table, an opening 8 by 12 inches is cut for the sink. The outer margin of the opening is formed by the side curb and angle iron frame (see Section CC).

At the head of the table two holes are bored for  $\frac{1}{2}$  inch water inlet pipes and at the foot of the table on the center line a hole is bored for a  $1\frac{1}{2}$  inch

\*From the Department of Pathology and Department of Maintenance of the Pittsburgh City Home and Hospitals.

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TABLE VI  
LOW XANTHOPROTEIN—LOW UREA NITROGEN—PATIENTS DISCHARGED

RACE AGE SEX	ADMISSION CLINICAL DIAGNOSIS	CONDITION	BLOOD			FINAL DIAGNOSIS
			URIC N MG %	CREA TININ MG %	XANTH	
1 W-25-M	Diabetic acidosis	Acutely ill	13		7	Acute appendicitis
2 W-50-M	Carcinoma of stomach		14		5	Carcinoma of stomach
3 W-64-M	Cystitis		18		4	Acute cystitis
4 W-37-M	Exacerbation of chronic nephritis		20		7	Chronic nephritis
5 W-62-M	Leucemia		11		7	Chronic lymphatic leucemia
6 W-27-M	Toxic hepatitis Neoarsphenamine		12		8	Arsphenamine reaction
7 C-48-M	Arteriosclerotic heart disease	Acutely ill	20		8	Syphilitic heart disease
8 W-51-M	Meningoencephalitis		20		6	Organic heart disease
9 W-52-M	Chronic nephritis		18		9	Coronary disease
10 W-61-M	Sacroiliac arthritis		18		7	Chronic nephritis
11 W-30-M	Functional neurosis		18		9	Sacroiliac arthritis
12 W-29-M	Cholelithiasis		12		9	Chronic cholecystitis
13 W-38-M	Peptic ulcer	Acutely ill	13		6	Gallbladder disease
14 C-39-M	Gonorrheal arthritis		14		6	Chronic cholecystitis
15 W-75-M	Arteriosclerotic heart disease		20		6	Acute arthritis
16 W-45-M	Lymphosarcoma		10		7	Coronary sclerosis
17 W-52-M	Hypertensive heart disease		16		6	Hodgkin's disease
18 W-31-M	Rheumatic heart disease		15		5	Hypertensive heart disease
19 W-62-M	Carcinoma of stomach		9		5	Rheumatic heart disease
20 C-60-M	Infectious nonspecific arthritis		14		4	Carcinoma of stomach
21 C-57-M	Generalized arterio sclerosis		25	1.8	7	Infectious polyarthritis and generalized arteriosclerosis
22 W-22-M	Exophthalmic goiter		12		9	Hypertensive heart disease
23 W-22-M	Hyperthyroidism		11		9	Thyrototoxicosis
24 W-53-M	Diabetes mellitus		10		6	Thyrototoxicosis
25 W-33-M	Acute articular rheumatism		16		7	Peptic ulcer and diabetes mellitus
26 C-48-F	Syphilitic aortitis		11		7	Rheumatic fever
27 W-46-M	Sciatica		10		5	Syphilitic aortitis
28 W-69-M	Hypertensive heart disease		20		6	Sciatica
29 W-41-F	Hypertensive heart disease		33	2.4	7	Hypertensive heart disease
30 W-36-M	Exacerbation Chr glomerulonephritis		21		8	Hypertensive heart disease
31 C-62-M	Syphilitic heart disease	Congestive failure	14		6	Acute glomerulonephritis
32 W-79-M	Chr myocarditis		17		9	Syphilitic heart disease
33 C-38-M	Hypertensive heart disease		10		8	Chr myocarditis and arteriosclerosis
34 W-53-M	Diabetes mellitus Hemiplegia	Congestive failure	10		7	Hypertensive heart disease
35 W-41-M	Acute articular rheumatism		13		7	Diabetes mellitus Hemiplegia
36 W-69-M	Arteriosclerotic heart disease		15		6	Acute articular rheumatism
37 W-55-M	Arteriosclerotic diabetes		15		10	Arteriosclerotic heart disease
38 W-66-M	Dyspepsia		18		6	Coronary thrombosis
39 W-48-M	Nontoxic adenoma of thyroid		9		5	Nervous dyspepsia
						Nontoxic adenoma of thyroid

shelf, and also serves as a support for the angle rod of the shelf. The bottom frame consists of two longitudinal and three cross members. The middle cross member also supports the main waste line. Four rubber-tired casters are attached to the lower frame directly under the corner legs.

All joints are bolted and the bolt heads in contact with the marble are countersunk. The bolts in the top frame were left loose until the marble top was set and then tightened. This action assures tight joints and locks the marble firmly in place. All iron work, plumbing, and the under side of the marble top were painted with white enamel. (For measurements and details see the general plan and sections.)

*Plumbing.*—Two 1½ inch brass drain pipes, one from the small sink and one from the screen at the foot of the table join at the center of the table and

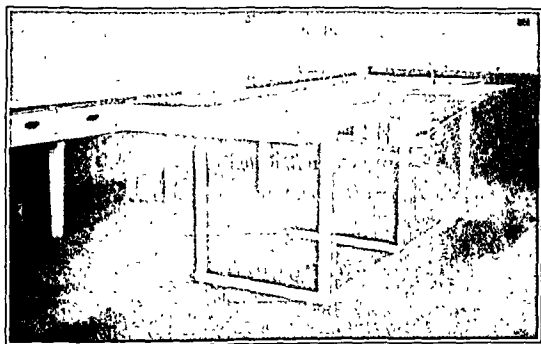


Fig. 2.—Photograph of completed autopsy table.

discharge over a floor drain equipped with a removable copper baffle ring to prevent splash. The drain from the sink is controlled with a 1½ inch valve (see Sections AA, BB, and CC).

The water supply lines are ½ inch red copper pipe except the nickel-plated gooseneck supply to the sink. The main supply begins at the foot of the table and extends along the center line to the head of the table. At the foot of the table the main line has two branches which are connected with rubber hose to hot and cold water supplies which are valved at the floor. A single branch off the main line supplies both inlets to the sink and each inlet is valved separately. The side inlet to the sink is equipped with a check valve to prevent syphoning. The main line terminates at the head of the table in two 1½ inch bell inlets with fan-shaped apertures which spray water over the surface of the table. All pipes are secured to the under side of the top angle frame with clamps and bolts. All valves are turned to the outside of the table for ease of operation (see Sections AA, and BB).

TABLE VII—CONT'D

POST-MORTEM NO.	RACE AGE SEX	PREMORTEM BLOOD CHEMISTRY		POSTMORTEM BLOOD		HOURS DEAD	POSTMORTEM DIAGNOSIS AND COMMENTS
		UREA N. MG. %	CREATININE MG. %	XANTH.	INDICAN MG. %		
29. 566	W-60-M	34	2.2	4		6	Marked eccentric hypertrophy of heart
30. 594	C-37-M	144	11.0	1	2.5	16½	Malignant nephrosclerosis
31. 599	C- 5-M			3	2.0	19	Arsenic poisoning
32. 598	C- 7-M			4	2.5	12	Heat prostration (clinically)
33. 600	W-67-M	18		2	0.3	2	Carcinoma of prostate
							Bilateral ascending pyelonephritis
34. 606	C-46-F	9	140.0	1	0.4	18	Chronic glomerulonephritis
35. 632	C-63-M	33	2.2	7	0.6	1½	Glandular hypertrophy of prostate gland

TABLE VIII

BODY FLUIDS

RACE AGE SEX	ANATOMIC DIAGNOSIS	BLOOD		PERICARDIAL FLUID						HOURS DEAD
		XANTH.	INDICAN MG. %	UREA N. MG. %	CREATININE MG. %	SUGAR MG. %	CHLORIDES MG. %	XANTH.	INDICAN. MG. %	
1. C-40-F	Fibrinous pericarditis	1	2.5	172	9.6	-	-	1	2.5	19
2. W-48-F	Hydropericardium	5	0.05	23		133	-	7	0.05	2
	Hypert. l. ventricle of heart									
3. C-38-M	Ancient myomalacia	6						5		15
4. W-54-M	Suppurative leptomeningitis	1						4		5½
5. C-54-F	Carcinoma of cervix	1		Peritoneal fluid, Xanth. 1				1		15
6. C-38-F	Malignant nephrosclerosis	1		Pleural fluid, Xanth. 4				1		16
7. W-86-M	Encephalomalacia of pons	4		31	2.3	96	764	6		1½
8. C-50-F	Carcinoma of pancreas	1		Pleural fluid, Xanth. 4				3		2
9. W-39-M	Periportal cirrhosis of liver	1		Peritoneal fluid, Xanth. 2				1		9
10. W-60-M	Eccentric hypertrophy heart	4		84	5.0	30	660	4		6
11. C-37-M	Malig. nephrosclerosis	1	2.5	233	15.7	78	560	1	0.45	16½
12. W-67-M	Carcinoma of prostate gland	2	0.3	71	5.0	80	735	4	0.4	2
13. C-46-F	Chronic glomerulonephritis	1	0.35	168	10.0	36	585	1	1.0	18

## SUMMARY AND CONCLUSIONS

Evidence is presented in this paper to confirm the findings of Becher and other investigators who reported very high value of the xanthoprotein reaction in cases of marked renal insufficiency either in uremia or approaching

so as to permit the free flow of blood. The lower end of this angle tube projects into the bottle for about 20 mm below the rubber stopper. The second hole in the rubber stopper accommodates a straight glass tube plugged with cotton. Its lower end extends only to the level of the lower end of the rubber stopper. After covering the angle tube with a small test tube the entire apparatus is sterilized by steam under pressure. After sterilization one places 30 to 50 cc

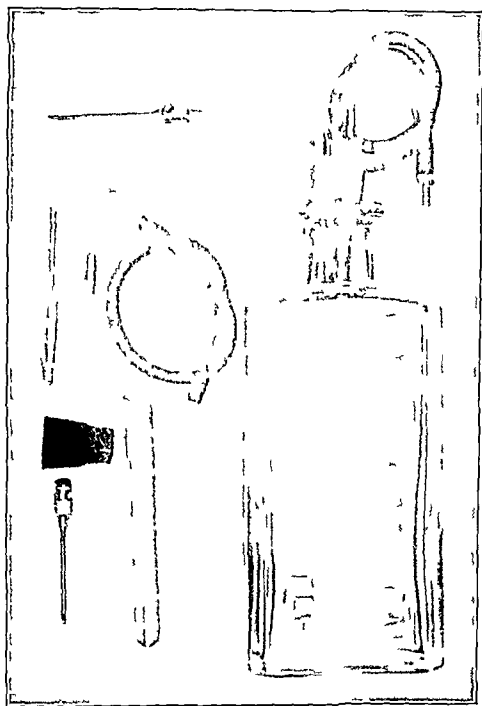


Fig 1—Bleeding bottle assembled ready for addition of the citrated saline solution. The separate small parts are shown at the left: (1) Curved inlet tube with tip ground to fit the needle; (2) straight outlet tube plugged with cotton; (3) rubber suction tube with glass mouth piece; (4) rubber stopper with two holes; (5) needle gauge 15 and (6) protective test tube.

of sterile 5 per cent sodium citrate in 0.9 per cent saline in the bottle best by means of a Pasteur bulb pipette. A small firm rubber suction tube is attached to the straight outlet tube and a No. 15 gauge needle sterilized by boiling in oil is fitted to the ground end of the angle inlet tube.

The donor is placed in a recumbent position and a garter tourniquet<sup>1</sup> is applied to his upper arm so as to cause maximum distention of the veins of the

7. Becher, E., Litzner, S., and Taglich, W. Der Phenolgehalt des Blutes unter normalen und pathologischen Verhältnissen, *Ztschr. f. Klin. Med.* 104: 182, 1926.
8. Becher, E.: Welche diagnostischen Schlüsse lassen sich aus starken Verschiedenheiten im Ausfall der Indikan und Xanthoproteinprobe ziehen? Eine neue Einteilung der Uramin, *München med. Wchschr.* 77: 432, 1930.
9. Boeminghaus. Über den Wert der Xanthoproteinreaktion für die Beurteilung der Nierenschädigung bei chronischer Harnstauung, *Ztschr. f. Urol.* 20: 881, 1926.
10. Goldman, M., and Burniewicz, J.: Über den klinischen Wert der Xanthoproteinreaktion im Blut, *Ztschr. f. Klin. Med.* 107: 716, 1928.
11. Gromer, V.: Zur Anwendung der quantitativen Bestimmung des Xanthoproteinwertes des Blut-serum nach Becher als Methode für den Nachweis der Niereninsuffizienz, *Zentralbl. f. Inn. Med.* 472: 1125, 1926.
12. Hoesch, K. Chemische Blutwerte bei echtem Koma und komaähnlichen Zuständen Leberkranker, *Ztschr. f. Klin. Med.* 117: 175, 1931.
13. Inoue, H. Klinische und experimentelle Untersuchungen über Xanthoproteinreaktion des Blutes bei Leberschädigungen, *J. Chosen M. A.* 23: 94, 1933.
14. Kammer, L.: Über die Xanthoproteinreaktion im Blut-serum, *Ztschr. f. Klin. Med.* 125: 632, 1933.
15. Melly, Bela.: Über den Wert Xanthoproteinreaktion im enterischen Blut in der urologischen Chirurgie, *Ztschr. f. Urol. Chir.* 24: 581, 1928.
16. Monas, B., and Shapiro.: The Value of the Indican Determination in the Blood in Cases of Renal Insufficiency, *Arch. Int. Med.* 45: 573, 1930.
17. Peters and Van Slyke. Quantitative Clinical Chemistry, Baltimore, 1931.
18. Rakestraw, N. W.: A Quantitative Method for the Determination of Phenols in Blood, *J. Biol. Chem.* 56: 109, 1923.
19. Rakestraw, N. W.: Chemical Factors in Fatigue. II. Further Changes in Some of the Blood Constituents Following Strenuous Muscular Exercise, *J. Biol. Chem.* 56: 121, 1923.
20. Rathery, F., and Waitz, R.: Réaction Xanthoprotéique dans les Affections Renales, *Compt. rend. Soc. de Biol.* 103: 214, 1930.
21. Rose and Alving: Personal Communication.
22. Scherk, G.: Über die klinische Verwertbarkeit der Xanthoproteinreaktion im Serum bei Nierenkrankheiten, *Med. Klin.* 23: 133, 1927.
23. Smith, M. I.: The Estimation of Tissue Phenols, *Pub. Health Reports* 48: 1487, 1933.
24. Theis, R. C., and Benedict, S. R.: Phenol and Phenol Derivatives in Human Blood in Some Pathological Conditions, *J. Biol. Chem.* 36: 99, 1918.
25. Tomietti. Importanza della determinazione della sostanze aromatiche de sangue nelle malattie renali, *Policlinico (sez. med.)* 36: 163, 1929.
26. Tschulow, K.: Über den klinischen Wert der Xanthoproteinreaktion (X P R) im Blute, *Wien. Arch. f. inn. Med.* 18, 19: 67, 1929/30.
27. Widenhorn, H.: Die Xanthoproteinreaktion, die Urochromogenreaktion und die Kaolinprobe als Nierenfunktionsprüfungen, *Ztschr. f. Urol. Chir.* 25: 215, 1928.
28. Wuhrman, F.: Reststickstoff und Xanthoproteinreaktion im Agonal und Leichenblut, *Ztschr. f. Klin. Med.* 127: 499, 1935.
29. Zamyslova, K.: Die Bestimmung des Indicans und der Xanthoproteinreaktion im Blute als Methode der Nierenfunktionsprüfung, *Ztschr. f. Klin. Med.* 116: 732, 1931.

desired, further doses may be introduced at once through the same needle after exchange of syringes. However, this is ordinarily undesirable, for the chief advantage of the method is due to spacing out of the doses. Upon withdrawal of the needle, pressure is applied immediately to the puncture, and while the arm remains extended, the pressure pad is held in place by a garter tourniquet lightly applied for a full five minutes. The same vein may be used again after an hour or so. By using the arms alternately one may give the desired amount of blood in several injections at intervals of thirty minutes or more, as convenient, without risk of too sudden dilatation of the vascular system of the patient and without danger of other unexplained reactions. Even the rare allergic patient who may react badly to the most precisely matched blood will take the small doses at intervals without disaster, and by employing even smaller doses, administered still more slowly, even the mildest reactions may be avoided.

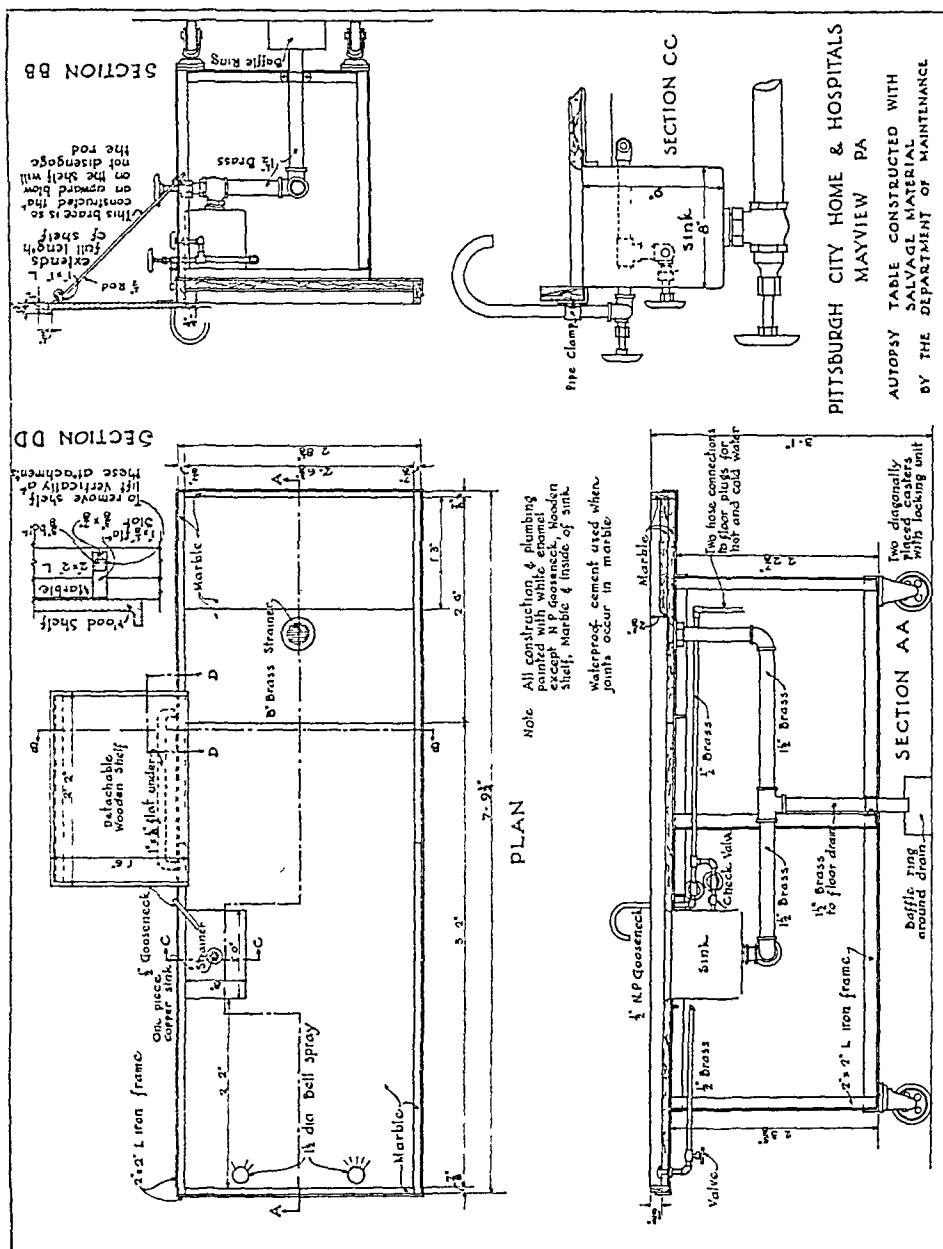
The disadvantages of the method relate particularly to the time consuming labor involved in the cleansing and meticulous sterilization of the glassware and needles and the necessity for the operator to be available to inject the blood many times during the day. The advantages relate especially to the welfare of the patient who escapes all dangers of ill effects due to the rapid dilatation of his vascular system and is also spared the psychic shock of the first transfusion by the usual methods. To many very sick patients the repeated intravenous injections, performed at the bedside, are of little more significance than hypodermic medication. The method is also highly elastic in various respects. One may separate the blood into constituent layers and may at will give the patient blood plasma or the sedimented corpuscles suspended in saline solution. Such separation may be particularly desirable when one is employing immunized donors.

In our hands, the method has been especially valuable in the supportive treatment of patients suffering from severe sepsis and particularly in cardiac and pulmonary diseases in which the immediate behavior following larger transfusions has sometimes been disappointing. Daily multiple intravenous injections by this technique have been continued for months without serious injury to the veins.

#### REFERENCE

1. Straub, Margaret E., and Mac Neal, Herbert P. A Tourniquet for Intravenous Procedures, *Arch. Dermat. & Syph.* 29: 717, 1934.

waste line. The waste is fitted with a 3-inch brass strainer countersunk flush with the surface of the table (see the general plan and Section AA).



**Table Base and Frame.**—The table is supported by an angle iron frame constructed of 2 by 2 angle iron  $\frac{1}{4}$  inch thick. The top frame consists of three longitudinal and six cross members, and is connected to the bottom frame by five vertical legs. One leg is placed on the left side of the table under the detachable

In addition to the elimination of contamination, this procedure possesses the virtues of economy and easy portability. It may be carried in the bag in any position, for this flask is not harmed by being carried on its side or inverted. This is in sharp distinction to the cotton stoppered containers where any deviation from the upright spells disaster. This is quite an advantage to those who like me, may be frequently called upon to take blood cultures at the bedside away from the hospital. Another readily apparent advantage is that men remote from immediate laboratory facilities can obtain cultures in this container, as no great technical skill is required and mail the container to the laboratory.

It has many advantages over its nearest competitor, the media laden vacuum tube with needle attached. It is far less fragile. The cost is far less and the difficulty of obtaining blood through the broken, constricted neck of the vacuum

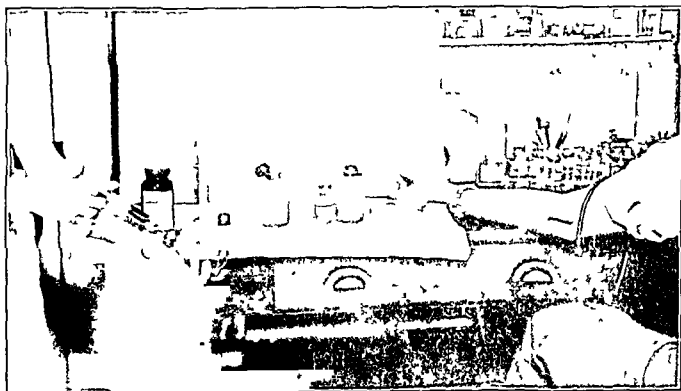


Fig. 1

tube is eliminated. Not to be overlooked, also, is the frequently cut finger from the fragments of glass at the point of fracture of the glass neck.

#### SUMMARY

1 A simple, economical, practical and "fool proof" method designed to facilitate the making of blood cultures is described.

2 A rubber cap is utilized and the blood culture is inoculated and withdrawn in the same manner in which vaccines and serums are obtained from their containers, i.e., through the rubber cap.

3 Being easily portable and safely carried in any position, the container may be carried about in a bag and mailed in when required.

4 As no special technical skill is required, our method places blood cultures at the disposal of the physician regardless of how remote his location may be.

The sink is made of sixteen gauge copper, bright on the inside. The outside is painted white. Its dimensions are 8 inches wide, 12 inches long, and 9 inches deep. A flange  $\frac{3}{4}$  of an inch wide around the top perimeter is locked between the bottom of the marble top and angle iron frame to hold the sink rigidly in place (see Sections AA, and CC).

*Detachable Wooden Shelf.*—The shelf is made of white pine painted with white enamel on the under side and orange shellac on the top. The shelf slopes slightly from the outer edge to the inside edge which projects  $\frac{1}{4}$  of an inch beyond the curb. Although the shelf is easily removed, its attachments are so constructed that it will not tilt and cannot be accidentally disengaged (see Sections BB, and DD).

*Cost of Materials—1936:*

Marble for top	\$ 28.00
Angle iron	5.60
Pipe valves and fittings	19.00
Copper sink	1.40
Casters, rubber-tired	12.00
Paint	4.00

Total material	\$ 70.00
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<i>Cost of Labor—1936:</i>	85.00
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Total cost	\$155.00
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#### REFERENCE

1. Forbus, W. D.: An Autopsy Table: A New Design, Arch. Path. 14: 506, 1932.

#### FRACTIONAL TRANSFUSION\*

WARD J. MAC NEAL, M.D., AND MARGARET E. (STRAUB) NEIL, NEW YORK, N. Y.

THE transfusion of a large quantity of compatible blood is ordinarily a Bland procedure when the recipient has just suffered a profuse hemorrhage and is otherwise in good condition. When, however, there is an extensive pneumonia, myocardial deficiency, septic thrombosis, or endocardial vegetations, the sudden introduction of a large volume of blood or other fluid into the vascular system may be followed by unfavorable or even disastrous results. We have found that these difficulties may be avoided by using citrated blood and injecting small amounts at intervals during the day, a method which we designate as fractional transfusion.

The blood from the donor is received into a suitable bottle of about 350 c.c. capacity. This bottle is fitted with a rubber stopper perforated by two glass tubes. One of these is bent at a right angle and its tip† is ground to fit a standard Luer needle. The lumen at the tip is drilled to a diameter of at least 1.5 mm.

\*From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

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†The ground glass tips to fit Luer needles can be obtained from Eimer and Amend, Third Ave. and 18th Street, New York City, and probably from many other dealers in glass apparatus.

specially constructed rectal applicator. The temperature difference is registered by means of a d'Arisson galvanometer. One half of the light beam reflected by the galvanometer mirror is directed toward a translucent scale and the other half strikes a photographic paper mounted on a rotating drum. The details of the apparatus can be visualized by the cross section in Fig 1 to which the following description refers.

A wooden box  $B_1$  100 cm long 10 cm wide and 44 cm high was made to hold the instruments. The top and the long sides are removable. The bottom extends 23 cm beyond one end. A smaller box  $B_2$  holding the rotating drum  $D$  can be attached to this extension and will  $W$ . The galvanometer  $G$ , a low resistance Leeds and Northrup type P with a sensitivity 15 microvolts per mm at 1 meter, is mounted on the wall  $W$ . The telescopic light projector  $L$  with a  $\frac{1}{2}$  amp 4 volt line filament light bulb and focusing lens is placed in a suitable position and can be adjusted so that the light after being reflected by the galvanometer mirror is focused on the photographic paper. A mirror  $M$  is placed so that one half of the light beam is reflected and focused on a translucent scale

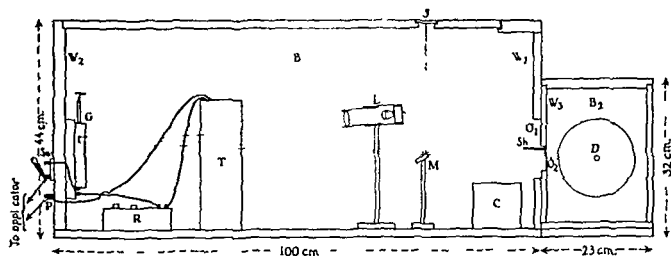


Fig 1—Apparatus for indicating and recording temperatures measured by a thermocouple applicator

$S$  placed on a narrow glass window in the cover. It is convenient to arrange this scale so that it can be moved laterally for zero adjustment. By means of this arrangement a reading can be taken at any time while the photographic registration is made. Openings  $O_1$  and  $O_2$  have been cut at the correct height in the adjoining walls  $W_1$  and  $W_2$  to let the light beam pass through. Box  $B_2$  is supplied with a shutter  $Sh$ , which can be pushed in front of slot  $O_2$  when no exposure of the paper is desired and the box can thus be made light tight and can conveniently be carried to the dark room for exchange of photographic paper. The drum  $D$  is rotated once in twenty four hours by means of a clockwork which is mounted on the outside of one of the walls of  $B$ .

A step down transformer  $C$  supplies current for the light bulb. A small light bulb attached to the outside of box  $B_1$  is in series with the resistance of the thermostat and is extinguished each time this resistance is shorted. The flashing of this bulb indicates that the thermostat is functioning correctly with an accuracy of about  $\frac{1}{100}^{\circ}\text{C}$ . The thermostat has been in continuous use for more than a year with no trouble. A resistance box  $R$  is used to obtain suitable sensitivity and critical damping of the galvanometer.

forearm. The skin is prepared with iodine and alcohol. Then the needle is passed into one of the large veins at the elbow, preferably with its point directed toward the wrist. The blood should flow in a stream into the bottle so as to fill it in two to four minutes. Suction hastens the flow, and this is also favored by steady muscular movement of the patient, closing and opening his fist. After filling the bottle, the donor's arm is dressed and, after a brief period of observation, he is dismissed to duty.

The citrated blood in the bottle is immediately mixed, best by drawing part of it up into a large bulb pipette and discharging this again into the original bottle. One then transfers the blood to large sterile tubes, each containing about 50 c.c., and these are stored in the refrigerator ready for use. Such blood keeps for several days, but we have not had occasion to use it after the fourth day. The corpuscles tend to become gummy so that the older blood does not work so well in the injection syringe. It is unnecessary to warm the blood before injecting it into the patient.

For injection into the patient, we use syringes of resistance glass, capacity 50 c.c., sterilized by steam under pressure. Large Pyrex glass tubes are used to protect the syringe, one for the barrel of the syringe and a slightly narrower one for the plunger, such that these two tubes will telescope later to hold the filled syringe. One attaches a No. 15 needle to the syringe, draws in 15 to 20 c.c. of sterile saline and withdraws the plunger to the end of the barrel so as to spread a complete film of saline solution between the barrel and plunger. The saline solution is left in the syringe to mix with the blood, 40 to 45 c.c., which is drawn in rapidly through the large needle. This needle is then replaced with a smaller one, ordinarily gauge 24, for injection into the patient's vein. An operator with powerful hands may be able to use even a 27 gauge needle but this usually offers so much resistance to passage of the blood that it is not recommended. Some operators will prefer a 22 gauge needle. The smaller needle does less damage to the patient's vein.

Ordinarily we insert the needle directly into the prepared arm of the patient and into the properly distended vein without difficulty. Penetration of the vein can usually be recognized by the experienced operator through the sense of touch. If the injection is started while the needle is outside the venous lumen, this error becomes manifest by increased resistance and the formation of a small rounded swelling (hematoma) under the palpating finger, a mishap which requires immediate withdrawal and application of moderate pressure. The beginner, and also sometimes the experienced operator in a difficult case, will prefer to puncture the patient's vein with a suitable needle (gauge 24) attached to a small syringe of saline solution and to assure himself of successful penetration of the vein by withdrawal of a little blood into the syringe or, when the patient's blood pressure is so low that this fails, by injecting the saline solution into the vein. When the position of the needle is satisfactory, it is then grasped firmly by forceps and held in position while the small syringe is replaced by the large one containing the blood. This procedure ordinarily requires the cooperation of an intelligent assistant but can, if necessary, be done by one person working alone. The blood is injected in a period of two to five minutes. If

soldered by a short application of a hot soldering iron. The small hole is sealed by the solder and any excess of material may be smoothed off with a file.

One wire leading from the applicator is threaded\* through a rubber tubing about  $\frac{1}{8}$  inch outside diameter, the other wire is threaded through a good quality rubber tubing, about  $\frac{1}{4}$  inch inside diameter. "Gas" tubing is suitable for the latter. The small tubing is then threaded through the larger tubing and pushed against B, the rod, A, penetrating into it. Shellac and many turns of strong thread wound over the junction of the rod and small tubing adequately seal the two wires from each other and hold the tube in place, the larger tubing is slipped over the applicator body for about  $\frac{1}{2}$  inch, the end of the rubber

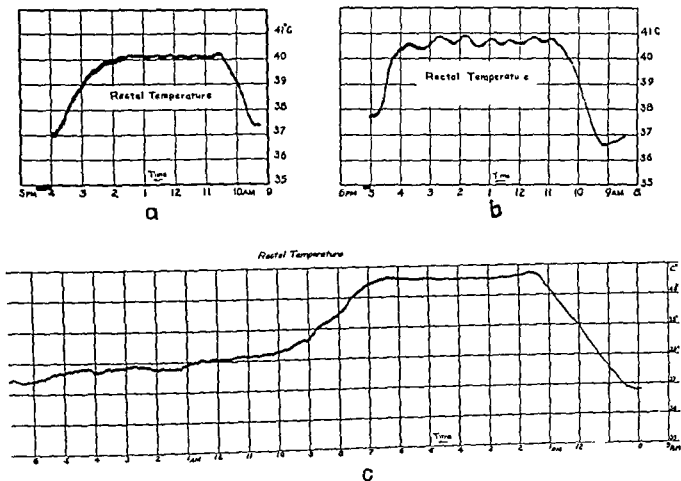


Fig 3—Photographic temperature records. A and B are for patients treated in a Kettering machine. C is for a patient treated by diathermy.

tubing is beveled so that thread may be wound to form a smooth junction between the applicator and tubing. The junction is shellacked.

Critical damping as well as suitable sensitivity of the galvanometer can be obtained by selecting proper parallel and series resistances. It is evident that the scale has to be calibrated in degrees. The thermostat is first set for a suitable temperature, e.g., 35° C (95° F). When the switch is open the light image should be directed to a proper line of the scale which then corresponds to 35° C. The rectal applicator is placed in water contained in a thermos bottle together with a calibrated thermometer and a few readings are taken at various temperatures within the range to be used. As the deflections are directly proportional to the temperature change over the range considered, only a few points on the

\* A material may be quite easily threaded through a rubber tubing by first drawing through a piece of stiff wire (music wire is good) which may be used to pull through other materials.

## A NEW AND SIMPLIFIED BLOOD CULTURE TECHNIC\*

J. M. FEDER, M.D., ANDERSON, S. C.

I HAVE struggled with every known method devised for obtaining a culture of the blood for the past twenty years and the results have been as variable as the means. Some were simple, some relatively efficient, some cumbersome, and others only a question mark, but all shared the same fault—undue handling of blood and media with consequent exposure to contamination. In the method described this main objection has been eliminated, while other desirable features have been added.

The method is simple. Kracke's media are employed for all routine work. The container is simply a six-sided, four ounce Pyrex nursing bottle, and its closure is the cap intended for that purpose, the Davol soft rubber nursing bottle cap.

We prepare our media in the usual manner as recommended by Kracke; 5 gm. of dry media, obtained from the Digestive Ferments Company of Detroit, is introduced into a container with 50 c.c. of distilled water. The flask is loosely plugged with gauze, and the cap is fastened to the neck with a rubber band. These are now sterilized at 15 pounds' pressure for twenty minutes. They are allowed to cool in the autoclave. Then with sterile-gloved hands the plugs are removed and discarded, and the tight-fitting rubber caps are stretched into place.† A large amount can be made at one time, as this media keeps indefinitely.

We keep a supply of 10 c.c. sterile syringes in large test tubes, the needles being protected from dulling on the bottom of the container by being encased in a section of glass rod slightly longer than the needle. This syringe together with the loaded, rubber-capped tube completes the armamentarium of the technician en route to the ward to obtain a blood culture.

The patient's arm is prepared in the usual manner. Ten cubic centimeters of blood is withdrawn and the top of the rubber cap having been either scrubbed with alcohol or painted with tincture of iodine is pierced with the needle and the blood expelled into the media and well shaken. No preliminaries are engaged in. Incubation is started at once. When subcultures are desired or examinations are to be made to determine presence of growth, material for the purpose is obtained in the same manner as when inoculated; through the cap. Two cubic centimeter hypodermic syringes are kept on hand for this purpose, sterilized in small test tubes, and the needle is simply thrust through the freshly asepticized cap and the desired amount withdrawn.

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†We have found that the rubber caps will go into place more easily if they are filled with water before sterilization. This water-filled cap is simply pressed into place.

In order to secure the required partial vacuum, we have found it necessary to open the sterilizer while still hot and immediately close the tubes. The required vacuum will be noted by the dimpling inward of the rubber cap.

## A STAIN FOR URINARY CASTS\*

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WILLIAM MUEHLBERG MD CINCINNATI OHIO

THE importance of urinary casts as prognostic evidence of cardiovascular renal disease has probably been neglected by the medical profession and the whole subject of these bodies appears to merit further study. Addis<sup>1</sup> has called attention to this point in his important contributions to the subject of this group of diseases and by his classification of Bright's disease he has fully demonstrated the value of a detailed study of urinary sediments.

Life insurance statistics indicate that casts either hyaline or granular, and either intermittent or constant herald the onset of cardiovascular renal disease about ten years before the cardiovascular renal apparatus is seriously affected. In other words, when casts are found in the urine the death rate appears normal during the first ten years but after the tenth year the death rate is about 63 per cent above normal and the excessive deaths are largely accounted for by organic diseases of the heart, cerebral apoplexy and Bright's disease. Evidently the age at which casts are first found is not of much significance. These statistics are based on a study of periods covering a total of 52,436 years, and their reliability appears more probable because two independent companies, with the material about equally divided between them obtained almost parallel results.

A technique for staining urinary casts is described in this paper in the belief that it may prove useful in further study of the structure and composition of these bodies.

Various stains for casts have been suggested from time to time especially iodine, picric acid, methylene blue, carmalum, and eosin. We have found no reference in the literature to the use of methyl blue for this purpose. This dye, however, especially in combination with picric acid brings out the detailed structure of casts and cast like bodies in a remarkable way. It does not furnish a differential stain since all of the mucous material is also stained blue. It serves, however, to bring the casts and cast like bodies into prominence and to reveal unsuspected details in their structure. The stain also makes photography of these bodies possible. The addition of eosin produces a contrasting color in many of the cellular structures of the urinary sediment.

With the technique described below hyaline casts are stained a clear blue of varying intensity. The more irregularly shaped bodies sometimes classed as cylindroids, are similarly stained. An irregular distribution of material, a "mealy" structure or a striated appearance sometimes becomes evident in bodies which appeared perfectly homogeneous before staining. Mucous threads and amorphous mucous material are also stained blue. They are clearly differentiated

\*From the Laboratory of the Union Central Life Insurance Company, Cincinnati and Northwestern University Medical School, Chicago.  
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## CONTINUOUS REGISTRATION OF RECTAL TEMPERATURE DURING TREATMENTS IN THE HYPERTHERM\*

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THE inducement of artificial fever or hyperpyrexia has become a widely used method of treatment for patients suffering from general paresis, gonorrheal arthritis, and a few other diseases. The five methods commonly used consist in: (1) wrapping the patient in blankets and waterproof material such as rubber sheets, (2) wrapping the patient as in the first method but speeding up the process by means of diathermy treatments, (3) using short-wave diathermy, (4) enclosing the patient in a box and supplying heat by means of light bulbs, and (5) placing the patient in a large box in which moist air is heated by means of electrical resistances and circulated by a fan as is done in the Kettering hypertherm. Whichever method is used, it is of utmost importance to watch the temperature of the patient carefully. In most instances this is done by means of an ordinary rectal thermometer which has to be inserted and removed at short intervals. This is a very inconvenient method. A considerable amount of warm moist air escapes during the process and a fluctuation in the temperature may thus be produced. A certain delay in obtaining the temperature is inherent in this method and that may occasionally lead to complications.

By means of electrical devices for measuring temperatures, it is possible to read the temperature at any moment without delay and without disturbing the patient. Such devices are not suitable when short-wave diathermy is used, but for all the other methods of inducing heat it is practical. We have found the thermocouple method very satisfactory, but a resistance type thermometer may be used instead. A suitable apparatus can easily be assembled after the few necessary instruments have been obtained. The initial expense and effort are well rewarded by the simple and satisfactory manner in which the temperature fluctuations can be followed. A physician in charge of treatments of this kind is unwilling to give up this method after once it has been tried. By means of a few simple additions and special arrangements it is possible to obtain continuous photographic registration of the temperature. The complete self-recording apparatus, which has been constructed here, will be described. The simplification which can be made if the photographic recording is unnecessary is self-evident.

A copper—constantan (or advance)—thermocouple is used. One junction is kept at constant temperature in a thermostat constructed in accordance with the description of Harry Clark.† The other junction is usually placed in a

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†J. Exper. Med. 35: 385, 1922.

side the envelope the cast may appear with typically rounded ends, it may be convoluted or folded back on itself or it may present segments of material more or less disconnected from each other.

The usual classifications of these bodies are probably too simple. On the other hand, some of the distinctions frequently made in the interests of morphologic classification appear to have little justification. For instance, an examination of these stained urinary sediments makes it difficult to believe that there is any reason for a sharp differentiation between the typical "cast," with rounded ends and parallel sides, and the thin bodies often called "cylindroids,"



Fig 1

which do not conform to this shape. Flat, striated mucous threads or ribbons are unmistakably distinct from either of these types but all gradations in shape and in structure can be found between the typical, homogeneous "hyaline cast" on the one hand and the long tailed loosely packed sometimes striated "cylindroid" on the other. The probable similarity of origin and significance of these bodies has been recognized<sup>4</sup> but in routine procedure an undue importance has often been attached to the "true cast."

Fig 1 shows photomicrographs of several different types of casts stained with this stain.

The thermostat is partly filled with water on top of which is poured a little oil to prevent evaporation. The constant temperature junction of the thermocouple is kept submerged in the water. The copper wire from this junction is connected to the resistance box *R* from which the circuit goes through the galvanometer and to switch *S<sub>c</sub>*. The copper wire leading from this switch to the rectal applicator should be taken from the same spool as the other copper wire. The constantan wire leads from the thermostat to the binding post *P*. There it is directly connected to another constantan wire which again must be taken from the same spool in order that an additional thermo-electric effect may be avoided. The size of both the copper and constantan wires used here is *B* and *S* No. 30 (0.25 mm. diameter).

A rectal thermocouple applicator that is not subjected to the high temperature and humidity of a Kettering hypertherm machine can be simply constructed by soldering a thermocouple junction to the inside of a hollow copper tip that is screwed to a bakelite tube of shape similar to that of Fig. 2. The thermocouple leads go through this tube, and inside a single outer tubing, to the cold junction and measuring instruments. Enamel insulation of the wires is sufficient, in these cases, to prevent the wires from short circuiting.

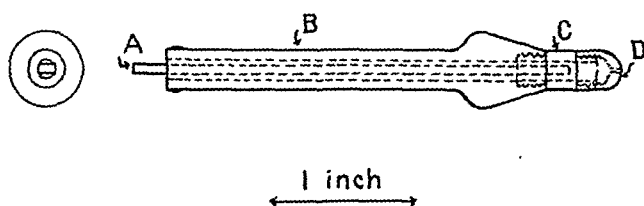


Fig. 2.—Rectal thermocouple applicator.

When the applicator is used in conjunction with a hypertherm machine, many difficulties are presented. Moisture and heat cause the insulation between the two wires to break down, forming additional parallel, high resistance junctions at points that are usually at high temperatures. Good thermal contact must be obtained between the thermocouple junction and the patient. (Fecal masses may thermally insulate the couple if the patient's temperature is changing rapidly.) The thermocouple wires must not be subjected to the mechanical stress imposed by restless patients. The applicator should be constructed so that the inside of the tube is hermetically sealed from the outside.

An applicator that has given good results is shown in Fig. 2. It consists of a copper collar, *C*, that is screwed into a bakelite tube, *B*. A copper tip, *D*, is screwed to the other side of the copper collar. Inside of the bakelite tube is pressed a small bakelite rod that had been filed flat on opposite sides. This rod should fit tightly into the tube and may be sealed to it by the application of a thin coat of shellac. It should be long enough to extend well into the copper collar, so that the thermocouple wires, which are kept separated by the rod, will have no opportunity for contact until they are at points of approximately the same temperature as the junction. In order to solder the couple to the tip, the enamel is removed from the wires just before they protrude from the small hole in the copper tip, a wet cloth is wrapped around the collar, and the wires are

## THE DETERMINATION OF CALCIUM IN CAPILLARY BLOOD\*

THOMAS M. VAN BERGHE, M.S. AND ROBERT M. HUNT, P.T.D., DENVER, COLO.

THE determination of the calcium content of the blood in infants is frequently desirable, but such determinations are rarely carried out in clinical work. Quantities of blood large enough for calcium determinations by the usual methods can only be obtained from the fontanel or external jugular vein. Methods which require only a few drops of blood from a finger prick have been developed<sup>1, 2</sup> but have been little used. In this paper results obtained with one of these methods, that of Nordbo,<sup>3</sup> are compared with those obtained with the Clark and Collip<sup>10</sup> modification of the method of Kramer and Tisdall,<sup>11</sup> and, using the Nordbo method, a comparison is made between the calcium values in blood obtained at the same time by finger prick and by venous puncture.

The method of Nordbo, chosen because of its simplicity, is a modification of that of Trevan and Bainbridge<sup>6</sup> which in turn is similar to the earlier methods of Huth<sup>4</sup> and of Lebermann.

*Methods*.—The procedure used by us is as follows. Six or seven drops of blood from a skin puncture are collected in a centrifuge tube containing 0.1 unit of heparin. Care must be used to avoid an excess of the anticoagulant.<sup>8</sup> After centrifuging, the plasma is drawn off and 0.1 cc. measured in a pipette calibrated "to contain." The pipette is washed with 0.1 cc. of water, and the washings added to the sample. Two or three drops of saturated ammonium oxalate solution are now added, and the tube is allowed to stand for at least two hours. The precipitate is thrown down in the centrifuge and the supernatant fluid is carefully drawn off. Approximately 1 cc. of 0.1 per cent ammonium oxalate is added as a washing fluid and the centrifuging is repeated. The supernatant fluid is once more removed and the tube is dried in an oven. When dry it is placed in a sand bath and the temperature is gradually brought to 200° or 250° C. By this treatment, the calcium oxalate loses its water of crystallization, and the excess ammonium oxalate is decomposed. After one half hour the temperature is brought to 450° C. The tube is heated at this temperature for ten minutes, and then removed, and cooled in air. At this high temperature, the oxalate is completely converted to carbonate.<sup>12</sup>

For the titration of the carbonate, 0.1 cc. of N/100 HCl is added to the calcium carbonate residue, and this is brought into solution by stirring with a fine glass rod. The tube is now heated for five minutes in a boiling water bath, and then titrated with N/100 NaOH until the solution reaches a pH of 4.6 as shown by the indicator, bromeresol green. The endpoint is determined by com-

\*From the Department of Biochemistry, University of Colorado School of Medicine.  
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scale need to be determined in this manner. In order to obtain reference points, on the photographic chart it is only necessary to open the switch for short intervals at certain times. The dots thus obtained supply a time scale and the straight line drawn through them marks the  $35^{\circ}$  baseline. Fig. 3 *A* and *B* shows such complete records of the rectal temperature throughout the time the patients were kept in the Kettering hypertherm machine. These records are read from right to left. Fig. 3, *C* is a twenty-four-hour temperature record of a patient who was treated by diathermy while wrapped in blankets and rubber sheets. It is interesting to note that the temperature stayed well above normal throughout the night after the patient had been unwrapped. The sensitivity of the galvanometer had been regulated so that  $1^{\circ}$  C. corresponded to a deflection of about 2 cm.

The apparatus just described has proved to be of great value in connection with the hypertherm treatments. Any change in the rectal temperature of the patient is immediately reflected in the galvanometer reading and a glance at the scale gives the operator the needed information. The apparatus may get out of order, e.g., by the breaking of a wire. If that happens, it is fortunately easily discernible, and the ordinary rectal thermometer can be substituted until repair has been made.

We have described the apparatus at this time because of the need for such a device in connection with fever therapy. It was originally constructed for some special research problems. The apparatus can, of course, be used for studies of temperature fluctuations in different portions of the human body during twenty-four hours, and the photographic recording is of special value for such investigations.

NOTE.—A stranded copper wire with good rubber insulation can be substituted for the single copper wire and small rubber tubing. In this case the flattened bakelite rod should be omitted from the rectal applicator and the insulation of the copper wire should extend into the metal collar. We wish to thank Dr. Cook, of this hospital, for this suggestion.

*Discussion*—We feel that our results clearly establish the value of Noidbo's method for the determination of blood calcium is a chemical procedure. Not only is agreement found between results obtained on the same blood with Noidbo's method and the method of Clark and Collip but with Noidbo's method close agreement is found between the calcium in blood obtained by finger prick and by venipuncture. This agreement between the calcium values found in venous and capillary blood is similar to that found by Smirk<sup>1</sup> who reports corresponding

TABLE I

COMPARISON OF THE CALCIUM CONCENTRATION IN SERUM (METHODS OF CLARK AND COLLIP AND OF NOIDBO) WITH THAT IN CAPILLARY BLOOD (METHODS OF CLARK AND COLLIP AND OF NOIDBO)

BLOOD FROM PATIENT	(A) VENOUS SERUM METHOD OF CLARK AND COLLIP	(B) CAPILLARY SERUM METHOD OF CLARK AND COLLIP	(C) CAPILLARY BLOOD METHOD OF CLARK AND COLLIP	VARIATION BETWEEN (A) AND (B)	VARIATION BETWEEN (B) AND (C)
	mg. Ca per 100 cc	mg. Ca per 10 cc	mg. Ca per 1 cc	mg. Ca per 100 cc	mg. Ca per 100 cc
1	11.06	10.44	11.11	0.57	0.70
2	10.97	11	11.09	0.12	0.70
3	11.02	11	11	0.03	0.0
4	10.75	10.11	11	0.6	0.10
5	10.78	11	11	0.01	0.0
6	11.11	10.81	11.81	0.2	0.0
7	11.13	10.61	11.19	0.54	0.80
8	10.47	10.11	10.39	0.26	0.30
9	10.21	10.9	11	0.7	0.20
10	10.97	10.11	11.19	0.02	0.20
11	11.08	11.19	11.11	0.11	0.0
12	9.81	9.71	11	0.07	0.0
13	10.07	10.31	10.31	0.32	0.0
14	10.38	10.11	10.19	0.19	0.0
15	11.37	11.09	10.11	0.28	0.10
16	11.21	11.39	11.11	0.14	0.0
17	11.00	11.19	10.99	0.19	0.0
18	11.02	10.91	10.91	0.03	0.08
19	10.81	11.19	11.19	0.38	0.0
20	10.70	10.49	10.39	0.21	0.10
Average	10.819	10.78	10.896	0.251	0.204

Difference in averages of (A) and (B) = 0.03 mg. Ca per 100 cc

Difference in averages of (B) and (C) = 0.116 mg. Ca per 100 cc

values for iron and hemoglobin in blood obtained at the same time from these two sources. The conversion of the calcium oxalate to carbonate is apparently well controlled. Willard and Boldyreff<sup>11</sup> found quantitative conversion of 600 mg. of calcium oxalate to carbonate at 450° to 500° C. in one to two hours. It is not surprising, therefore, to find similarly complete conversion of such small quantities as 0.03 to 0.04 mg. at 450° C. in ten minutes. The single washing of the precipitated calcium oxalate with 0.1 per cent ammonium oxalate evidently does not introduce a significant error.

## SUMMARY

1. Blood calcium was determined by the method of Noidbo on 0.1 cc. of serum and by the method of Clark and Collip on 1 cc. of the same serum. The calcium values thus obtained agreed within the limits of experimental error.

2. Determinations by the Noidbo method showed that the calcium concentration in capillary blood obtained by finger prick is the same as that in venous

from the cast-like bodies by their structure. Granular material is usually stained darker. Mixed, finely granular casts present a striking picture of fine, dark granules powdered over the light blue, hyaline body. Some coarsely granular casts are stained deep blue, the granules of others are yellow, orange, or dark reddish brown. Renal epithelial cells are usually red, sometimes orange or yellowish. Red blood cells are stained a brighter red. Pus cells are usually red, occasionally blue. Epithelial cells from the urinary passages are either red or blue. Fat globules are unstained. In cells which are undergoing fatty degeneration, the fat globules are seen strikingly against reddish cellular material. The picric acid gives a light yellowish background.

Picric acid is used because when it is present the details of cast structure are clearer and the casts have a smoother, more natural appearance than in its absence. It has, however, the following disadvantages. (a) It complicates the microscopic picture by precipitating mucous material which is then stained. In our experience this almost never interferes with the study of casts, which stand out much more clearly than in unstained material.\* In fact, in an occasional sample containing bacteria and débris and apparently no casts when unstained, the addition of the stain has revealed the presence of numerous casts. (b) Picric acid acts antagonistically toward eosin so that some, though not all, of the eosin-staining elements appear orange or yellow rather than red in its presence. However, if not too much picric acid is added the red color predominates in these bodies. The advantages of picric acid outweigh its disadvantages.

The use of this stain has suggested possible differences in structures otherwise undifferentiated. For instance, variations in the intensity with which "hyaline" or mixed casts are stained suggest differences either in consistency or in chemical composition. We have found casts containing renal epithelial cells imbedded in a "hyaline" matrix which take the stain more slowly and less intensely than the typical hyaline casts. On the other hand, some "hyaline" casts take the stain more deeply than usual. We are inclined to agree with Emerson<sup>2</sup> that a large group of casts exists which are more distinct and solid looking than hyaline casts but would not be classed as "waxy." It seems probable that in such casts there is a greater condensation of material than in the typical hyaline casts and that all gradations in such condensation can be found. The different staining reactions of coarsely granular casts and of pus and epithelial cells, mentioned above, may have to do with different stages in the disintegration of cellular material.

Often the stain reveals the presence of a lightly stained, mucus-like envelope, apparently covering the cast material. This envelope is often clearly seen at the end of a cast which has apparently been squarely broken off, or between two square-ended segments which appear to be in the process of separating from each other. The "tails" of the so-called cylindroids often appear to be the ends of these envelopes which may or may not contain thinned-out cast material. In-

\*We have occasionally found a sample in which the casts had a shaggy appearance due possibly to the precipitation by the stain of large amounts of amorphous material. Reference to the unstained material and staining with a solution of methyl blue in water instead of picric acid are desirable in such cases. They do not occur frequently.

A second magnet is used as a release. When energized, this magnet attracts its armature, which releases the detent which holds the ratchet wheel. With this detent released, the ratchet returns to the original position through its mechanical spring. The release magnets are wired in parallel so that contact with the reset key causes a synchronous return of all indicators to their original position. These magnets, armatures, pawls, detent, and ratchet remain in the same position and relationship as in the original telephone selector. The wiring

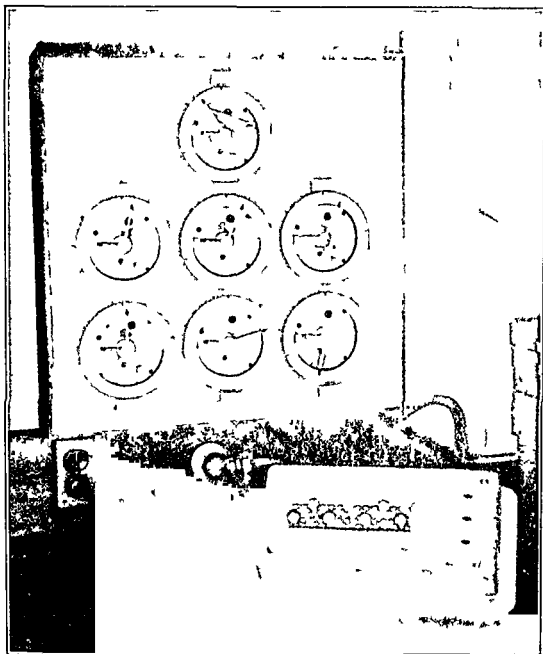


Fig 1

alone is changed. An arrow is soldered to the contact brush which completes the indicator arm. A third small magnet, one of the original five, is wired in parallel with the selector magnet to an independent circuit which is connected with the master indicator thereby giving the total count. The master indicator is similar in construction to the individual indicators with the exception that when the count has reached one hundred a contact arm closes a bell circuit.

The seven indicators are mounted in a box faced with wall board, the master indicator at the top. The diameter of the individual face is five and one-

## STAINING TECHNIC

*Solutions.*—

1. A 0.5 per cent solution of eosin in water.
2. 1 c.c. of a 1 per cent aqueous solution of methyl blue added to 10 c.c. of saturated picric acid. The picric acid does not have to be particularly purified. We have used Merek's Picric Acid Reagent. About 10 drops of glycerol should be added for each 10 c.c. of picric acid.\*

*Method.*—The urine is centrifuged and the supernatant liquid decanted as usual for microscopic examination. One drop of the eosin solution is added to the sediment and mixed by side to side shaking. After a minute or two 2 drops of the methyl blue picric acid solution are added and mixed. The color of the sediment should be distinctly blue green. If it is reddish brown, more of Solution 2 should be added until the blue green color is obtained, but too much is to be avoided. Some of the stained sediment is then transferred to a slide, covered with a cover glass, and examined microscopically. The amounts of the two stains added may be varied according to the amount of sediment present and to the particular character of the sediment. More eosin may be added if the cells have not been stained sufficiently red. Enough of the methyl blue should be present to stain the casts a distinct blue, but too much will stain them too dark.

More permanent slides may be made by adding more glycerol to the sediment and sealing the edges of the cover glass with vaseline or balsam.

We are indebted to Mr. George O'Connor, of the Union Central Life Insurance Company Laboratory, for his assistance in the study of this stain, and to Mr. Frederick Riehle, of the same Laboratory, for the photographs, taken with the photomicrographic apparatus which he constructed, with some modifications, according to the directions of Kennedy.<sup>5</sup>

We also wish to thank Professor Chester J. Farmer, of Northwestern University Medical School, for his suggestions in regard to this work.

## REFERENCES

1. Addis, T.: A Clinical Classification of Bright's Diseases, J. A. M. A. 85: 163, 1925.
2. Emerson, C. F.: Cylindruria, J. A. M. A. 46: 5 and 89, 1906.
3. Endman, H. B.: The Origin of Tube-Casts, J. A. M. A. 59: 1952, 1912.
4. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis, ed. 8, 1935.
5. Kennedy, J.: Making Your Own Photomicrographic Camera, Scientific American 152: 76, 1935.

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\*Instead of methyl blue solution, 1.5 c.c. of blue black fountain pen ink (Waterman's, Carters, Skrip, etc.) added to 10 c.c. of saturated picric acid may be used. The dye used in these inks is evidently similar to methyl blue. The results with ink solutions are almost as good as those with methyl blue.

mg per 5 cc of blood. The calcium contents of these different plasmas were determined by the acidimetric micromethod of Nordbo<sup>10</sup> and by the permanganate titration method of Clark and Collip. Since the values obtained were in good agreement the averages only are reported. The results are summarized in Table I.

Heparin 1, when present in quantity about ten times the amount necessary to prevent coagulation reduces the calcium value about 40 per cent, Heparin 4 in the same weight concentration reduces the calcium value 12 to 15 per cent, while Heparin 2 shows a tendency to raise the calcium value slightly. Heparin

TABLE I  
THE INFLUENCE OF HEPARIN UPON THE DETERMINATION OF BLOOD CALCIUM

HEPARIN IN 5 CC OF BLOOD			0.0	0.01	0.1	1.0	2.0	4.0	10.0	16.0	20.0
			(cent M)								
Calcium content of plasma in mg per 100 cc	Heparin 1	Blood 1	10.35			10.15	10.06	7.70	6.46		
	0.7 unit per mg	Blood 2	10.08			9.77			5.80		
	Heparin 2*	Blood 1	12.00			11.99	12.30	12.20	12.45		
	0.0 unit per mg	Blood 2	11.26			11.76	12.09	12.30	12.39		
	Heparin 3	Blood 1	10.85			11.35	11.18	11.34	10.56		
	0.5 unit per mg	Blood 2	11.33					10.10	10.79	10.0	9.60
	Heparin 4	Blood 1	11.55			11.09	11.00	11.19	9.80		
	0.6 unit per mg	Blood 2	10.45					11.39	9.09	8.34	7.89
	Heparin 5	Blood 1	10.79			11.59	11.89	12.39	13.19		15.28
	1.2 unit per mg										
	Heparin 6	Blood 1	11.79			12.00	12.00	12.00	12.40		12.10
	1.2 unit per mg										
	Heparin 7	Blood 1	10.08	9.98	9.98	10.99			11.20		
	100 units per mg										

\*This sample had very little activity. A small clot appeared in all but the last tube in Blood 1. In Blood 2 1 mg per 5 cc of Heparin 1 was added to each tube.

samples 5 and 6 (Hynson, Westcott and Dunning) though more active than the samples made in our laboratory, cause increases in the calcium value when used in excess. When 20 times the minimal quantity of Heparin 5 is used (20 mg), an increase of 40 per cent in the calcium value is produced. The increase caused by 1,000 times the minimal quantity of Heparin 7 (10 mg) is only 12 per cent. With the exception of Heparin 2 all of the samples made in this laboratory, when used in excess, reduce the calcium value. All four samples are student preparations and were made at about the same time. The three made by the method of Howell (1, 2, and 4) are identical in appearance, but though Heparin 1 is fairly active, Heparin 4 is less active and Heparin 2 has practically no activity.

**Discussion.**—In evaluating these data it must be remembered that, with the exception of Heparin 7, the active substance is a very small part of the bulk of the preparation. It is quite possible that the effect on the calcium determination is produced by material which is inert so far as blood coagulation is concerned. This is almost certainly true in the case of a rise in the calcium value, as occurs with Heparin samples 5 and 6, for there is no relation between this rise in calcium and the amount of active heparin present. When an increase in calcium follows the addition of heparin to a blood sample, we feel that calcium must have been present as an impurity in the reagents used in its preparation. When a fall in calcium occurs, this effect seems to be proportional, at least roughly, to the

parison, against a white porcelain background, with a buffered standard at pH 4.6 containing the same amount of bromeresol green as is used in the unknown.

For throwing down the precipitates, use is made of ordinary 15 c.c. centrifuge tubes, which have been drawn out to a finer tip by heating in a Bunsen flame and allowing the tip to stretch out vertically when soft. The sand-bath for the incineration is about 8 cm. deep. The tube containing the dry calcium oxalate is thrust into it vertically so that its tip touches the bottom. A gas-filled mercury thermometer\* capable of recording 550° C. is also thrust to the bottom beside the tube. Accurate titration is accomplished with a modified Rehberg buret,<sup>9</sup> having a capacity of 0.2 c.c.

In working with our subjects, all of whom were adults, we found it necessary to scrub the finger tip with a brush, using soap and water, before puncturing it. Otherwise, high calcium values were obtained apparently as a result of contamination of the blood with material from the skin.

Calculation of the calcium present in serum may be made by the following formula:

$$\text{mg. of Ca in 100 c.c. of sample} = (\text{c.c. of exactly } 0.01 \text{ N HCl} - \text{c.c. exactly } 0.01 \text{ N NaOH}) \times 200$$

*Experimental.*—The Nordbö technic was first checked on solutions of calcium chloride. Using 0.1 c.c. quantities of a carefully prepared calcium chloride solution containing 9.81 mg. of calcium per 100 c.c., the following yields in mg. of calcium per 100 c.c. were obtained:

9.70	9.50	9.90	9.31
10.09	9.70	10.09	9.70
9.70	9.70	9.11	10.09

Average = 9.72

The errors are somewhat larger than those reported by Nordbö (not more than 3 per cent), but certainly not large enough to invalidate the method as a clinical procedure.

Calcium was determined by the method of Nordbö on both venous serum and finger-prick plasma, obtained at the same time, and these were checked on the venous serum sample by the technic of Clark and Collip.<sup>10</sup> Table I summarizes the results of calcium determinations in a series of bloods from twenty normal subjects. The greatest variation between the venous serum calcium values on any one sample by these two methods was 0.84 mg. per 100 c.c. of serum with an average variation of 0.251 mg. The difference in the average calcium content of the twenty samples as determined by the two methods was 0.039 mg. per 100 c.c. of serum. A similar comparison of venous serum calcium (Nordbö method) and that of capillary plasma shows a maximum variation of 0.80 mg. per 100 c.c. of the sample with an average variation of 0.204 mg. The difference between the average of the twenty samples of capillary plasma and the twenty samples of venous serum was 0.116 mg. per 100 c.c. of the sample.

\*Scientific Materials Company of Pittsburgh, Pennsylvania.

## REFERENCES

- 1 Graisheimar, E M and Arnold, A W Blood Chemistry Changes in Children Produced by Exposure to the Alpine Lung, *Am Rev Tuberc* 14 479 1926
- 2 Holt, G W Heparin in Blood Calcium Analysis, *Proc Soc Exper Biol & Med* 29 315, 1912
- 3 Cantarow, A Calcium Metabolism and Calcium Therapy, Philadelphia, 1931 Lea and Febiger, p 56
- 4 Loucks, M M, and Scott, F H Calcium in the Coagulation of the Blood, *Am J Physiol* 91 27, 1929
- 5 Clark, E P, and Collip, I B A Study of the Titill Method for the Determination of Blood Serum Calcium With a Suggested Modification *J Biol Chem* 63 461 1925
- 6 Kramer, B, and Tidball, F F A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J Biol Chem* 47 475, 1921
- 7 Howell, W H Heparin, an Anticoagulant, *Am J Physiol* 63 434 1922
- 8 Charles, A F, and Scott, D A Studies on Heparin I The Preparation of Heparin *J Biol Chem* 102 425, 1933
- 9 Scott, D A and Charles A F Studies on Heparin III The Purification of Heparin *J Biol Chem* 102 437, 1933
- 10 Nordbo, R Mikro Calciumbestimmung im Blute, *Biochem Ztschr* 246 410 1932

## A DIABETIC KIT\*

H E FAVER, M D, BUFFALO, N Y

FOR some time it was felt that the necessity for a compact and portable diabetic kit existed. To be useful, the kit should be small self contained and the outfit should include an insulin syringe, a needle Benedict's solution and facility for sterilizing syringe and needle and for testing the urine. Various types of electric heaters were tried, and as most laboratory manuals stated that the Benedict's solution should be boiled vigorously during the test, the tube containing this solution was heated directly by the electric element. In every case this direct heating of the tube caused violent bumping and expulsion of the solution. Various methods were tried to control this such as wire gauze surrounding the tube, bits of wire gauze within the tube, glass beads and small pieces of agate without success. Another factor that militated against the usefulness of an apparatus which heated the test fluid directly was the fact that the syringe and needle could not be satisfactorily sterilized in this manner and that other provisions had to be made to keep the needle and syringe continually immersed in alcohol.

It was then decided to test the efficiency of Benedict's solution when the tubes were immersed in boiling or simmering water and kept at a temperature of approximately 200 degrees F for various periods. To have a control in testing this method it was decided to construct a small cylindrical boiler electrically heated, which would contain from one to four small test tubes, and use it in routine urine tests. It was decided to test this boiler in the laboratory of the Buffalo State Hospital. The routine urines would be subjected to two tests, one by

\*From The Buffalo State Hospital

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blood. Hence, values obtained by the determination of calcium in blood from these two sources are directly comparable.

#### REFERENCES

1. Nordho, R.: Mikro-Calciumbestimmung im Blute, *Biochem. Ztschr.* 246: 460, 1932.
2. Groak, B.: Mikro Calciumbestimmung im Serum und Plasma, *Biochem. Ztschr.* 212: 47, 1929.
3. Fairhall, L. T., and Howard, R. G.: A General Method of Quantitative Microchemical Analysis, *J. Roy. Microscop. Soc.* 53: 129, 1933.
4. Hirth, A.: Le Dosage du Calcium dans le Plasma Sanguin, *Compt. rend. Soc. de biol.* 88: 458, 1923.
5. Lebermann, F.: Ueber eine neue klinische Methode der Mikrokalziumbestimmung im Blut-serum, *Munchen. med. Wchnschr.* 71: 1392, 1924.
6. Trevan, J. W., and Bainbridge, H. W.: The Estimation of Calcium in Blood-Serum, *Biochem. J.* 20: 423, 1926.
7. Rappaport, F., and Rappaport, D.: Volumetric Method for Determining Calcium in 0.2 Cubic Centimeter of Serum, *Mikrochemie* 15: 107, 1934.
8. Van Bergen, T. M., and Hill, R. M.: The Effect of Added Heparin on Calcium Determinations in Blood Plasma, *J. Lab. & Clin. Med.* 22: 862, 1937.
9. Longwell, B. B., and Hill, R. M.: A Modified Rehberg Burette for Use With Titrating Solutions Which React With Mercury, *J. Biol. Chem.* 112: 319, 1935.
10. Clark, E. P., and Colp, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.
11. Willard, H. H., and Boldyreff, A. W.: The Determination of Calcium by Ignition of Calcium Oxalate to Carbonate in Air, *J. Am. Chem. Soc.* 52: 1898, 1930.
12. Smurk, F. H.: The Effect of Water Drinking on the Blood Composition of Human Subjects in Relation to Diuresis, *J. Physiol.* 78: 127, 1933.
13. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J. Biol. Chem.* 47: 475, 1921.

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### A HOMEMADE ELECTRIC COUNTER FOR DIFFERENTIALS\*

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SIDNEY C. DALRYMPLE, M.D., NEWTON LOWER FALLS, MASS.

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WITH the modernizing of many hospitals, the house telephone is being replaced by the standard telephone system.

The electrical units of the house system can be utilized to advantage in making a blood counter for differentials. Such a counting apparatus (Fig. 1) was made in our hospital machine shop from a disused autophone system at practically no expense.

In the house system the connections between stations are made and maintained by a series of selectors each of which contains five magnets. By utilizing two of these magnets, the indicator for the individual count is controlled.

The indicator arm, which is the contact brush of the phone system, is attached to a ratchet which, in turn, is controlled by a selector magnet through its armature and pawl. The contact made in the key box causes the magnet to become energized and attract its armature, which causes the pawl to engage the ratchet and step it around one tooth for each attraction.

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\*From the Pathology Laboratory of the Newton Hospital  
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boiled at the same time. In the construction of the boiler, it was decided to decrease the length of time within which water was brought to a boil, as in the ones that heated more rapidly, there was some spluttering of water. It was found that if the water was kept slowly simmering results were quite satisfactory.

The cooperation of Ilmer F. Sevrin,haus of the University of Wisconsin, in the early development of this instrument is gratefully acknowledged. Also the cooperation of L. M. Green, pathologist at the Buffalo State Hospital is gratefully acknowledged for the performance of the laboratory tests.

## THE EXTRACTION OF CHOLESTEROL FROM BLOOD\*

H. J. ROSE, AND CECILIA RILGEL, PHILADELPHIA, PA

THE desirability of a uniform method of analysis for free and combined cholesterol in both bile and blood led to a study of the applicability of the digitonide method described by us<sup>1</sup> to three methods of extraction of whole blood, serum, or plasma. The methods of extracting cholesterol from blood were (1) Blood<sup>2</sup> alcohol ether extraction, (2) Schoenheimer Speiry<sup>3</sup> alcohol acetone extraction, (3) direct extraction of the whole blood, serum, or plasma with ether and magnesium sulphate, as described for bile in a previous paper.<sup>1</sup>

In both (1) and (2) the extraction is carried out as described by the authors. Subsequently the digitonin precipitation is made on a suitable aliquot.

In (3) 1 cc of ovalated whole blood or plasma (or serum) is diluted to 10 cc with water. One cubic centimeter of 15 per cent magnesium sulphate is added, and the mixture then shaken vigorously for twenty minutes with three successive 100 cc portions of ether. The ether fractions are decanted into a 500 cc Erlenmeyer flask, the ether distilled off to a small residue (1 to 3 cc) and the residue taken up in alcohol acetone (1:1) as in the bile analysis.

TABLE I

SAMPLE	DILUTION	EXTRACTIONS	CHOLESTEROL	
			TOTAL	FREE
	CC		MG %	MG %
Human whole blood	3 to 100	Alcohol Ether	184	104
Human whole blood	5 to 100	Alcohol Ether	190	104
Human whole blood	5 to 50	Alcohol Acetone	185	103
Human serum	2 to 25	Alcohol Acetone	215	---
Human serum	1 each	Multiple Ether	217	-
Dog whole blood	5 to 100	Alcohol Ether	144	104
Dog whole blood	5 to 50	Alcohol Acetone	152	104
Dog whole blood	1 each	Multiple Ether	149	99
Dog plasma	5 to 50	Alcohol Ether	123	48
Dog plasma	5 to 50	Alcohol Acetone	121	48
Dog plasma	1 each	Multiple Ether	121	44

\*From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania.

Received for publication August 28, 1936.

half inches and can be read at a distance of five feet. The wires to the keyboard are contained in a six-foot movable cable, allowing perfect mobility of the keyboard. The number of individual selectors required will depend upon the white cell classification used. Dry cells are employed to operate the selectors. The apparatus shown in the figure has been in use for four months and has proved most satisfactory, reducing the time in making counts by nearly one-half.

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## THE EFFECT OF ADDED HEPARIN ON CALCIUM DETERMINATIONS IN BLOOD PLASMA\*

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THOMAS M. VAN BERGEN, M.S., AND ROBERT M. HILL, PH.D., DENVER, COLO.

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WHILE investigating methods for the determination of calcium in very small quantities of blood, it became evident that it would be preferable to use plasma rather than serum. There are two advantages in using plasma: (1) a greater volume of plasma can be obtained; (2) there is a shorter interval between the drawing of the blood and the time when the analysis may be carried out. If plasma is to be used, an anticoagulant must be chosen which does not influence the analysis. Heparin would seem to be an excellent anticoagulant for this purpose if it fulfills this condition. A review of the literature fails to answer the question. Greisheimer and Arnold,<sup>1</sup> and Holt<sup>2</sup> report higher values for calcium in heparinized plasma than in serum; Cantarow,<sup>3</sup> in a series of 100 determinations, reports lower values for calcium in heparinized plasma than in serum; Loucks and Scott<sup>4</sup> found that heparinized plasma gave results that did not differ from those obtained on serum more than the experimental error of the method employed. All of these investigators used the Clark and Collip<sup>5</sup> modification of the Kramer and Tisdall<sup>6</sup> technic. Because of this lack of agreement, we felt that this question should be reinvestigated.

*Experimental.*—In our study seven different samples of heparin were used. Four were prepared in this laboratory; two were from different lots prepared by Hynson, Westcott and Dunning, Inc., Baltimore, Maryland; and one, of high potency, was prepared in the Connaught Laboratories, Toronto, Canada.† We have no information on the method by which the commercial samples (No. 5 and 6, Table I) were prepared. Of our samples, No. 1, 2, and 4 were prepared according to the method of Howell<sup>7</sup> from dog liver, and No. 3 by the method of Charles and Scott<sup>8</sup> from beef liver. Sample 7, from the Connaught Laboratories, was, presumably, prepared by the method of Charles and Scott,<sup>8</sup> and purified by the method of Scott and Charles.<sup>9</sup> The activity of the different samples was estimated by the method of Charles and Scott.<sup>8</sup>

Equal quantities of blood from one individual were added to a series of tubes containing dry, powdered heparin in amounts varying from 0.01 to 20.0

\*From the Department of Biochemistry, University of Colorado School of Medicine.  
Received for publication, August 8, 1936.

†This sample was kindly furnished by Professor D. A. Scott.

and is used in this case to trip the sweep circuit. The circuit of the multivibrator is shown in Fig 1. With the given values it vibrates approximately 200 to 800 times per minute. When all four condensers  $C_1$ ,  $C_2$ ,  $C_3$ ,  $C_4$  are in the circuit, the lower half of the range between 200 and 400 vibrations is obtained. By throwing the switches  $S_1$  and  $S_2$ , condensers  $C_2$  and  $C_3$  are disconnected and the upper range from 400 to 800 is covered. The variable resistances  $R_1$  and  $R_2$  which are on the same shaft serve to adjust the frequency within the two ranges mentioned above. The dial on these resistances is calibrated to read in number of vibrations per minute by using the 60 cycle A.C. as a standard.

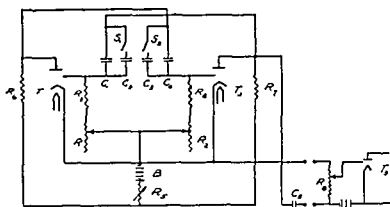


Fig 1

$B = 135$  volts  
 $C_1, C_2, C_3, C_4 = 0.1$  mfd  
 $C_5 = 0.004$  mfd  
 $R_1, R_2, R_3, R_4 = 200,000$  ohms  
 $R_5 = 10,000$  ohms

$R_6, R_7 = 20,000$  ohms  
 $R_8 = 100,000$  ohms  
 $T_1, T_2 =$  type 76 tube  
 $T_3 =$  discharge tube of sweep circuit

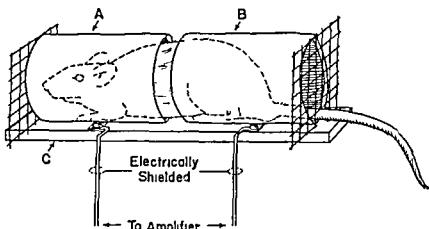


Fig 2

The apparatus is operated by batteries, this simplifies the setup and eliminates any possibility for the oscillator to lock with the A.C. due to the low frequency at which it operates.

Resistance  $R_5$  is used to compensate for any change in battery voltage and is adjusted once before each set of readings in the following manner. A.C. is connected to the vertical plates of the cathode ray tube and the dial set to a point where a multiple of the sine wave should be stationary. If the wave tends to drift it is brought to a standstill by means of  $R_5$ . It is essential that the frequency of the thyration circuit be lower than that of the relaxation oscillator, otherwise the sweep will return before it is tripped by the multivibrator.

activity of the heparin sample. The samples prepared in this laboratory arranged in descending order of their activity as anticoagulants (1, 4, 3, and 2) are found also to be in descending order<sup>1</sup> of their activity in decreasing the calcium precipitated from blood by oxalate. The preparation of a heparin which causes a fall in the calcium value was entirely fortuitous on our part, and no record of the reagents employed was kept. It seems possible, however, that our reagents were unusually free from calcium and that this is the reason the heparin prepared with them shows the properties reported here. The results obtained when Heparin 7 from the Connaught Laboratories was used closely approximate a balance between these two extremes. This sample produces neither a rise nor a fall in calcium when moderate amounts are used, and even with 100 times the minimal amount only a slight increase in the calcium value occurs. A consideration of the foregoing brings us to suggest that, in preparing heparin, if little or no calcium is present in the reagents, correspondingly little will be present in the product. Any excess of such a heparin used beyond that necessary to prevent clotting will remove calcium from the blood sample until it is "saturated." If just enough calcium is present in the reagents to "saturate" the heparin, then no matter how much excess of such a "saturated" heparin is used the calcium value will remain unchanged.

It is evident from the data in Table I that in most cases even two or three times the usual minimal quantity of heparin might be used without appreciable influence on the calcium value, but that in some cases (Heparin 5) there may be enough calcium contamination to make the sample unsuitable for use in such determinations. Any new sample of heparin to be used for this purpose should be tested by determining the effect of an excess on the determination of blood calcium.

All the samples of heparin tested with the exception of the relatively pure sample from the Connaught Laboratories, Heparin 7, contained such large amounts of inorganic phosphate as to render them unsuitable for use when phosphate is to be determined.

#### SUMMARY

1. The effect of excess heparin has been studied in the determination of plasma calcium.
2. Some samples of heparin used in excess produce a rise in the calcium value; others produce a fall.
3. When a rise is produced, it is apparently due to contamination of the heparin sample.
4. When a fall is produced, it appears to be related to the activity of the heparin preparation.
5. It is suggested that the difference in samples of heparin is due to differences in the calcium content of the reagents used in their preparation.
6. When heparin is to be used in preparing plasma for determination of calcium, the effect of adding an excess of the preparation should be tested.
7. Heparin, except when highly purified, cannot be used when phosphate is to be determined in the blood.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFF, M.D., ABSTRACT EDITOR

## LEAD POISONING, A Modified Stain for Stipple Cells in, Mc Kinney, R. A., and Rosenzweig, S. J. A. M. 106 1660, 1936

The three reagents required are kept for convenience in covered Coplin jars. The technique is as follows:

Fix dried smear in acetone free methyl alcohol for from three to five minutes and transfer directly to Wright's stain (staining time predetermined for each lot of stain used), wash in tap water and transfer to dilute ammonia water (25 cc of stronger ammonia water in 1,000 cc of distilled water), dip up and down rapidly until blue color runs from slide, wash in tap water, dry and examine.

The finely stippled or coarsely dispersed "basophilic aggregations" in the red blood cells appear distinctly black against the gray or pink of the stained cell. The white blood cells retain the usual nuclear stain.

The ratio of lead affected cells to the normal red cells is determined by an adaptation of the Fomon platelet counting technique. A minute opening in a paper disk dropped into the ocular diaphragm gives a suitable counting field. The stipple cells and the normal red cells in each field are counted, but tabulated in separated columns until 250 normal red cells have been counted. The number of stipple cells is then multiplied by 4, giving the ratio of such cells to 1,000 normal cells. The number of thousands of red blood cells per cubic millimeter multiplied by the number of stipple cells per thousand cells gives the approximate number of lead stipple cells per cubic millimeter of blood.

This staining method offers nothing fundamentally new, its chief advantage lies in the fact that a simplification and combination of two previous techniques makes possible:

1. A rapid detection and enumeration of stipple cells in a spread which is also adequately stained for a differential count of the white blood cells.
2. The utilization of reagents usually found in any physician's office or clinical laboratory.
3. A technique which stains dried smears two months old as satisfactorily as fresh dried smears.

## LYMPHOGRANULOMA INGUINALE, Cultivation of the Virus in Mice and Its Use in the Preparation of Frei Antigen, Grace, A. W., and Suskind, F. H. Arch. Dermat. & Syph. 33 853, 1936

A strain of the virus of lymphogranuloma inguinale obtained from the pus and glandular material of a patient presenting the inguinal type of the disease has been successively transmitted in mice by intracerebral inoculation for 41 passages (at the time of writing).

The virus increased in virulence with successive passages in mice.

Lymphogranulomatous mouse brains provided a readily available source of specific Frei antigen, the potency of which increased with successive passages.

Frei antigens prepared from mouse brains were tested six months after preparation and found to retain their potency for at least that length of time.

Emulsions of normal mouse brains prepared and tested in the same way as Frei antigen did not produce any appreciable reaction.

In the 143 Frei tests done on 22 lymphogranulomatous human subjects and the 145 tests done on 38 normal human subjects, no untoward effects were caused by the use of material prepared from mouse brains.

boiling the Benedict's solution with a gas flame in the orthodox manner, and the other test by immersing the test tube containing the Benedict's solution and urine in a simmering boiler. In the boiler the same quantity of reagents and urine was used and kept immersed in the boiler where the water was kept constantly simmering for a period of five minutes. The simmering water in all cases was at the level of, or slightly above, the Benedict's solution in the test tubes.

The results of the tests that were run in duplicate are shown in the appended table and consist of 88 urines; they were the routine urines that were examined

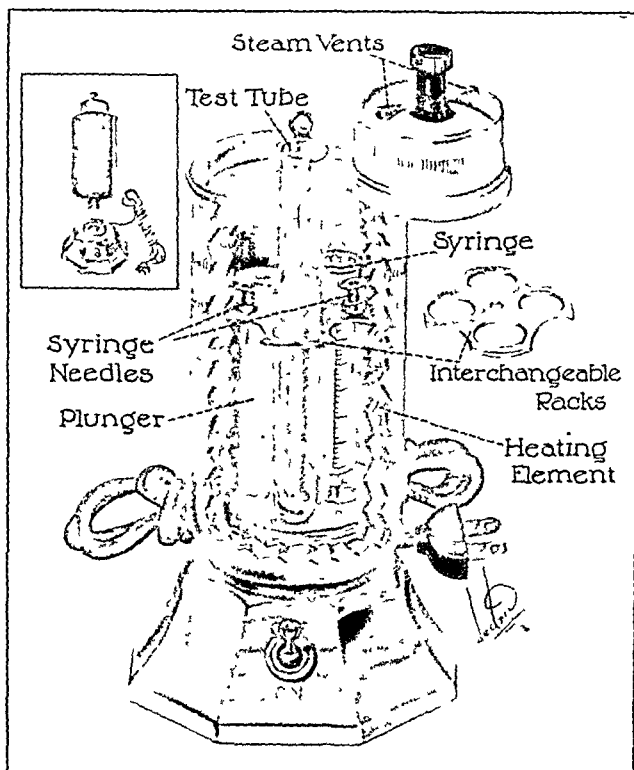


Fig. 1.

in the laboratory of the Buffalo State Hospital from April 30 to May 11, 1936, inclusive. The results in all cases were exactly identical, indicating that the reactions would go to completion if the tubes containing the Benedict's reagents and urine were immersed in simmering water at 200 degrees F., or more, for five minutes.

Of 88 urines tested routinely, 3 showed positive sugar in both the diabetic kit and in the usual manner of laboratory testing; 3 showed trace of sugar in both types of testing; and 82 were negative in both types of testing.

As the kit is constructed,\* it contains a small rack with four openings, so that 1 to 4 tubes may be tested at the same time, or 1 tube, syringe, and needle

\*This kit is constructed by the Gomco Surgical Manufacturing Corporation, 87 Ellicott Street, Buffalo, N. Y.

moderate increase after the operation but an increase appeared toward the middle or end of the first week, rarely before the third day.

In cases of hemorrhagic thrombocytopenia, in which the number of platelets had been much diminished, splenectomy was followed by an immediate rise in all but one instance. This rise was not maintained in a fatal case and was delayed in the case of a boy who has lived two and one half years after operation. The peak was reached, as a rule, in the second post operative week, and a level of 1,000,000 platelets per cubic millimeter was maintained for a week or for several months.

A fall in the reticulocyte count was characteristic of typical cases of congenital hemolytic icterus.

In patients with Cooley's anemia, the response of the platelets was most irregular in onset, number, and duration of the increase nor was it ever as high or of as long duration as in the other groups. Increase in the number of nucleated red cells is characteristic in these cases.

Operations other than splenectomy were not followed by any appreciable rise in the platelet count during the postoperative period of observation.

#### **TYPHOID FEVER, Rapid Slide Test for the Serological Diagnosis of, Welch, H., and Mickle, F. L. Am J Pub Health 26 248, 1936**

A rapid slide test for the diagnosis of typhoid and paratyphoid fevers is presented which requires approximately 4 minutes to complete. The results obtained in a study of 1,100 serums indicate that the slide test is at least as sensitive and specific as the tube tests with which it was compared. The stability of the antigens used, the speed and accuracy with which a serum may be analyzed by this method, and the consistency of the results obtained, indicate that the test should be an excellent one for use in public health laboratories and in hospitals where an early exclusion of typhoid fever in certain undiagnosed cases is often important.

The method is as follows:

On a 5 by 7 glass slide five rows of small wax rings are placed (made as in the conduct of Kline precipitation tests).

The patient's serum is pipetted in the following amounts: 0.08, 0.04, 0.02, 0.01, 0.005, and 0.002 ml in the first four rows, using a Kahn pipette (0.2 ml graduated in thousandths). In the fifth row (used only for the first test each day) 0.08 ml of 0.85 per cent salt solution is added to each of the four rings for controls on each of the four antigens. A drop of "O" antigen is added to each of the serum amounts in the first row and to the first ring in the fifth row. Similarly, a drop of "H" antigen is added to each of the serum amounts in the second row and to the second ring in the fifth row. *S. paratyphi* is added to the third row and *S. schottmuelleri* to the fourth row, each with the appropriate controls in the third and fourth rings, respectively, in the fifth row. The above serum antigen mixtures in each row correspond to dilutions of 1:20 through 1:640 in the tube test. All antigens are shaken gently but well before using. Each row of serum antigen mixture is thoroughly mixed with a separate toothpick or applicator starting with the smallest amount (0.002 ml) of serum and working from right to left. After mixing, the glass slide is gently rocked back and forth 15 or 20 times and the degree of clumping estimated. The type of agglutination obtained with the "O" antigen does not correspond to the typical small flaking or granular agglutination obtained in the tube test, and hence "O" and "H" agglutination cannot be differentiated by appearance. This is no disadvantage since both types of antigens are used.

#### **PHOSPHATASE ACTIVITY, Serum Calcium, Inorganic Phosphorus and, Guttman, A. B., Tyson, T. L., and Gutman, E. B. Arch Int Med 57 379, 1936**

The calcium and inorganic phosphorus content of the serum and the serum phosphatase activity were determined in 4 cases of hyperparathyroidism, 76 cases of Paget's disease, 6 cases of multiple myeloma and 45 cases of neoplastic disease of the bones. The relevant data from the determinations in the differential diagnosis of the diseases of bone under consideration are discussed.

For free cholesterol, digitonin precipitation is made directly on the alcohol acetone extract. For total cholesterol the extract is saponified by adding 0.1 c.c. of 33 per cent potassium hydroxide and heating two and one-half hours at 38° C. The extract is then acidified and precipitated with digitonin.

Subsequent analysis is as described for bile.<sup>1</sup>

Mean values are reported in Table I.

#### SUMMARY

Cholesterol, free and combined, is extracted from whole blood, plasma, or serum equally well by single extractions made with alcohol ether or alcohol acetone, or by multiple extracts with ether.

#### REFERENCES

1. Riegel, C., and Rose, H. J.: Determination of Free and Combined Cholesterol in Bile, *J. Biol. Chem.* 113: 117, 1936.
2. Bloor, W. R.: The Determination of Cholesterol in Blood, *J. Biol. Chem.* 24: 227, 1916.
3. Schoenheimer, R., and Sperry, W.: A Micro Method for the Determination of Free and Combined Cholesterol, *J. Biol. Chem.* 106: 745, 1934.

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## A METHOD FOR DETERMINING THE HEART RATE OF SMALL ANIMALS\*

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MICHAEL KNIAZUK, RAHWAY, N. J.

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THE counting of the heart rate of small animals with a frequency of more than 200 beats per minute cannot be accomplished accurately by auscultation or palpation, but requires special methods of recording. Usually an electrocardiogram is taken and the number of beats per minute calculated from the photographic record. This makes it impossible to obtain the results instantly; although the method is accurate, it is impractical for routine determinations on a large scale.

In the method to be described, the photokymograph, together with the electromagnetic oscillograph, is replaced by a cathode ray oscillograph. Visual observations of the electrocardiogram on the fluorescent screen of the cathode ray tube are made by connecting the amplified heart action current to the vertical deflecting plates and a sweep circuit oscillator to the horizontal plates. If the frequency of the sweep is the same as that of the heart rate, a stationary picture of the electrocardiogram will result, whereas a difference in frequency of the two currents will cause a drifting of the wave to the right or left. In this way readings of the heart rate can be made at the instant of synchronization, provided the frequency of the sweep circuit is known. The ordinary type of sweep circuit using a gaseous discharge tube is not sufficiently constant in its frequency output to be calibrated and used for this purpose. The relaxation oscillator, or multivibrator, is a much more constant frequency generator,

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\*From the Merck Institute of Therapeutic Research.  
Received for publication, August 11, 1936.

From further studies (Ibid 51 11 13 6) the same authors conclude that

Assuming, as it seems safe to do that the determination of fibrin gives a satisfactory index of the plasma fibrinogen, the experimental results obtained show that injections of the mother's whole blood do not increase the fibrinogen content of the plasma in the normal newborn infant. It seems improbable therefore, that the beneficial effect of such injections in the case of hemorrhagic disease of the newborn infant is due to increased formation of fibrinogen.

#### **BLOOD, Occult, in Stools of the Newborn Bonar B E Am J Dis Child 51 255, 1936**

Occult blood is found too frequently in the stools of newborn infants to be attributed to parental sources or to gross lesions of the alimentary tract.

Investigation of other possible etiologic factors reveals that it is not influenced by sex, frequency of stools, initial loss of weight (dehydration) or the hemoglobin and erythrocyte levels. It is not responsible for the characteristic reduction of the levels of these constituents of the blood during the first ten days of life.

Occult bleeding is influenced by the weight at birth, by the kind and amount of food to some extent by the duration of labor. An explanation is offered for the decreased amount of bleeding found in infants delivered by operation.

The observations of some authors that the incidence of occult blood in the stools is increased when jaundice is present could not be fully corroborated. The relationship noted in this study between bleeding and jaundice was found to be more apparent than real.

Prolongation of coagulating and bleeding times as well as the tendency for the blood of the newborn to hemolyze are definite contributing factors in occult bleeding. However, the bleeding seems to be more closely related to the bleeding time and hence to the condition of the vessel walls, than to the clotting elements of the blood.

Occult bleeding is probably more dependent on the early bacterial invasion of the alimentary tract than on the type of organism involved.

The observations recorded lend support to the previously mentioned assumption that the presence of occult blood in the stools is the result of hyperemia of the mucosa. However this explanation fails to take into consideration the fact that blood is detected before the irritation by bacteria or food can be responsible. The following mechanism which begins with birth, seems plausible. In the first two or three days the vascular engorgement from pressure and asphyxia during the birth process results in the loss of blood either by rupture of minute mucosal vessels or through increased permeability of their walls. In the next few days bleeding is increased temporarily by the intense hyperemia set up by bacteria and food. This is probably augmented by a prolongation of the clotting and bleeding times at this time and possibly by an increased permeability of the vessel walls. With the subsidence of some of these factors there is a corresponding reduction in the amount of bleeding, but this may be delayed somewhat by the rapid rise in consumption of food.

#### **UNDULANT FEVER Due to B Suis Horning, B G J A M A 105 1978 1935**

An outbreak of undulant fever with fourteen cases and three deaths occurred in a home for elderly persons.

Raw milk from the institution herd was the only source of infection found.

The herd consisted of thirty six cows and one bull. The blood from two cows was positive and from another suggestive for brucella infection.

*Brucella suis* was isolated from the blood of two patients and from an abscess of a third patient.

Blood was drawn from thirty two swine kept by the institution. Nine were positive and seven suggestive for brucella infection.

The cattle had opportunity for natural infection from the swine.

Epidemiologic evidence suggests that the outbreak was due to *Brucella suis* received from drinking raw milk from cows infected with *Brucella suis* from swine.

For taking the electrocardiogram, the animal is placed in a special holder (Fig. 2). The animal rests on two wet cloth pads *A* and *B* with his fore and hind legs. The pads are re-enforced internally with sheet metal and fastened to the bakelite strip *C*. The leads are not typical for electrocardiography but serve well the purpose of obtaining an action current of the heart. The cage must fit the animal snugly, and therefore different sizes are required for guinea pigs, rats, mice, etc.

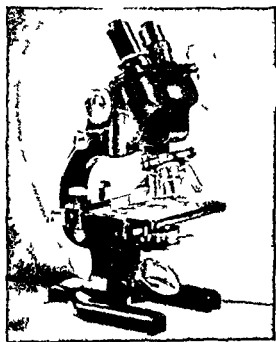
The principal advantages of the method are:

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3. By using instead of needle electrodes the special holder described, excitement of the animal is to a large extent overcome. This is especially important for securing consistent and accurate results in bradycardia tests.

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The brains of mice which died from an intracerebral inoculation of the virus of lymphogranuloma inguinale showed meningoencephalitis on histologic examination. Exudate into the meninges and ventricular system and perivascular infiltration with infrequent formation of abscesses in the brain substance were the chief lesions.

The inflammatory cells consisted of polymorphonuclear leucocytes, macrophages, plasmacytoid cells, a few small lymphocytes and a number of very large cells, possibly macrophages. The proportion in which the cells of the first three types occurred depended on the length of time that elapsed between inoculation and death.

Intracytoplasmic bodies were encountered infrequently.

**LYMPHOGRANULOMA, Frei Test With Antigens Made From Mouse Brain, Strauss, M. J., and Howard, M. E. J. A. M. A. 106: 517, 1936.**

Some change occurs in antigens made from mouse brain within a few weeks after preparation which, when injected intradermally, gives rise to a reaction almost indistinguishable from a true positive reaction.

The nature of this change is at present unknown.

This occurs in antigens made from the brains of normal mice as well as in antigens made from the brains of mice inoculated with lymphogranuloma inguinale.

The false reaction is induced in normal subjects as well as in patients with lymphogranuloma inguinale.

For this reason Frei antigens made from mouse brain would not appear to be suitable for the routine diagnosis of lymphogranuloma inguinale.

**BLOOD: Values for Red Blood Cells of Average Infants and Children, Mugrage, E. R., and Andresen, M. I. Am. J. Dis. Child. 51: 775, 1936.**

The quantity of hemoglobin, the number of red blood cells, and the volume of packed cells have been determined accurately on samples of venous blood from children ranging in age from birth to thirteen years.

The children are divided into eighteen groups according to age, and the mean values are reported for each group. Differences in blood values between boys and girls are not considered to be especially significant. Averages for the amount of hemoglobin, the number of red blood cells, and the volume of packed cells which are high at birth reach a low level in the period between the ages of two and four months. There is a transient rise between the ages of four and eight months, followed by a second decrease. The sustained increase begins at the age of eighteen months and continues to the twelfth year. Values for the twelve-year-old children are practically identical with those for the normal women of our previously reported series.

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The corpuscular hemoglobin concentration remains at a remarkably uniform level throughout the period of life covered in the present study. The majority of values for children of all ages fall within the range observed for the blood of normal adults.

**SPLENECTOMY, Blood Picture After, in Children, Wollstein, M., and Kreidel, K. V. Am. J. Dis. Child. 51: 765, 1936.**

In forty-four children the blood picture following splenectomy varied with the cause for the removal of the spleen.

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2. The calcium and inorganic phosphorus contents of the serum are within normal limits in cases of uncomplicated Paget's disease. The serum phosphatase activity is almost invariably increased in cases of advanced disease reaching values forty or more times the mean normal value. The level for serum phosphatase is roughly proportional to the extent of involvement of the bones and is probably affected also by the activity of the osseous lesions. In this sense, the determination may be of value in prognosis. Increased blood phosphatase activity is not specific for Paget's disease. The increase in instances of early, localized Paget's disease is often equivocal, so that the determination is of little value in such cases. Nor is it usually of assistance in differentiating cases of osteoplastic metastases in the bone, in which serum phosphatase activity is also increased.

3. Definite hypercalcemia has been observed in a number of cases of multiple myeloma. The inorganic phosphorus content of the serum is normal or somewhat elevated. The serum phosphatase activity is usually normal or slightly elevated.

4. Hypercalcemia is present in occasional cases of carcinoma with extensive, predominantly osteolytic metastases to the bones. The amount of inorganic phosphorus in the serum is usually within normal limits, but it may be depressed in cases of advanced cachexia or increased when renal insufficiency is present. The serum phosphatase activity is usually moderately increased or essentially normal.

In cases of carcinoma with extensive, predominantly osteoplastic metastases, the serum phosphatase activity may be as high as that in advanced Paget's disease. The content of calcium and that of inorganic phosphorus in the serum are normal, except when renal insufficiency is present. In cases of carcinoma with metastases to the liver, with or without jaundice, variable increases in serum phosphatase activity are observed.

**LEUKOCYTES, Rest and Activity Levels of, in Health and Disease, Medlar, E. M. Arch. Int. Med. 57: 367, 1936.**

Definite "rest" and "activity" levels of the leucocyte count do not exist if the activity is limited to mild exercise.

Complete relaxation, such as a night's sleep or two hours in bed, appears to bring about a more irregular distribution of the leucocytes, both in the total and in the differential count, than is present during a state of normal activity.

Great care should be exercised whenever an attempt is made to interpret the significance of the leucocytic picture when the fluctuations noted are within a normal range.

Fluctuations occur in abnormal as well as in normal leucocytic pictures.

These variations, when allowed for, do not seriously interfere with the pathologic interpretation of the significance of leucocyte counts.

**BLOOD COAGULATION, Factors Involved in, in the Newborn Infant: IV. Variations in Fibrinogen Content, Crane, M. M., and Sanford, H. A. Am. J. Dis. Child. 51: 99, 1936.**

The quantity of fibrin formed in the plasma of infants during the first ten days of life has been studied. The average value for fibrin under the conditions of this investigation was found to be 0.38 gm. per 100 c.c. of plasma, with variations between 0.22 and 0.67 gm. In the great majority of cases the content was between 0.25 and 0.55 gm. There is commonly a rise in the amount of fibrin during the first three to five days of life, after which there is no consistent change.

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Attention may also be called to the interesting paper‡ by P. Masucci and M. I. Moffat in which the loss of phenol and tricresol in biological products when stored at room temperature in rubber capped vials because of the diffusion of the phenol or tricresol vapors through the rubber caps was described.

K. George Falk

Dear Sir:

In connection with our article on Pulmonary Moniliasis which appeared in the April issue, p. 687, we should like to give credit to our laboratory technician, Rachel Milos, for the routine laboratory work done in this connection.

Earl L. Warren.

---

\*This JOURNAL 22 641, 1937

†Page 642

‡J. Am. Pharm. Assn. 12 117, 1923



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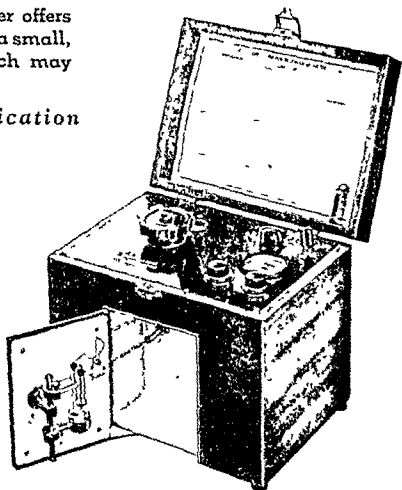
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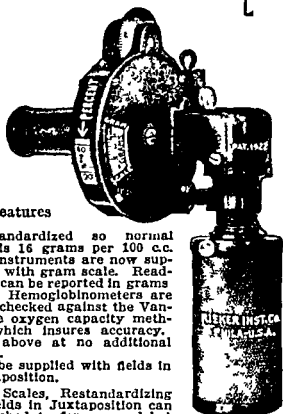
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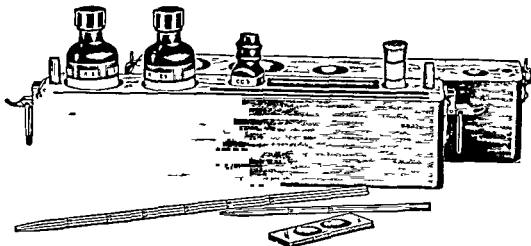
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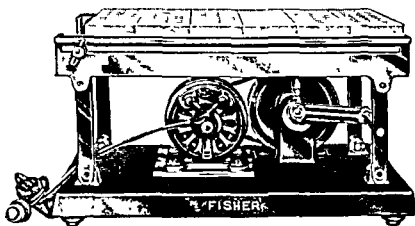
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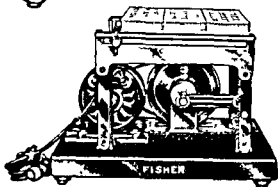
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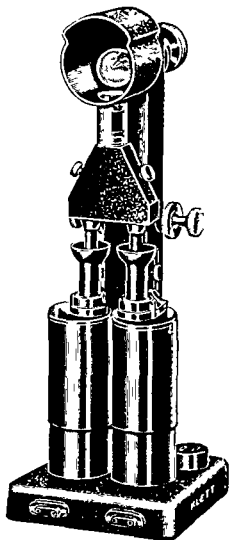
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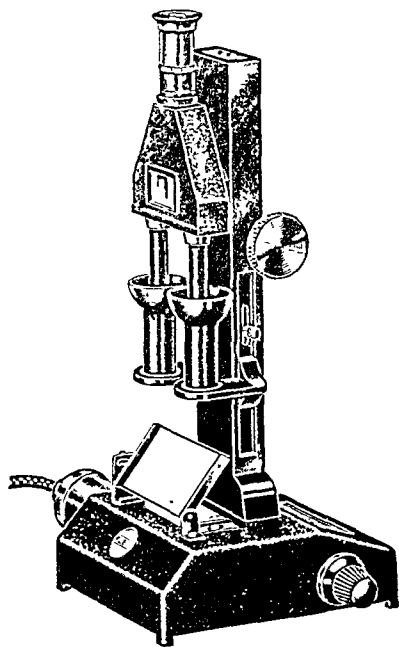
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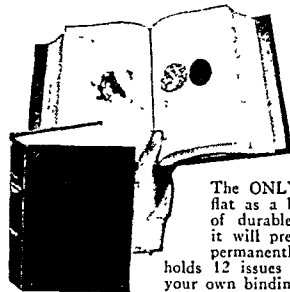
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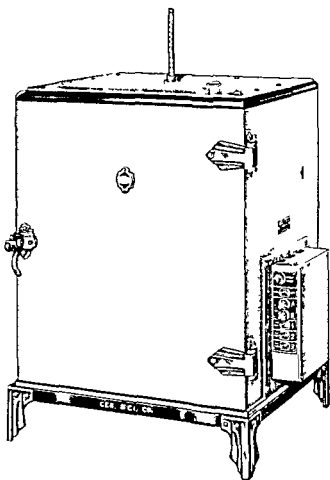
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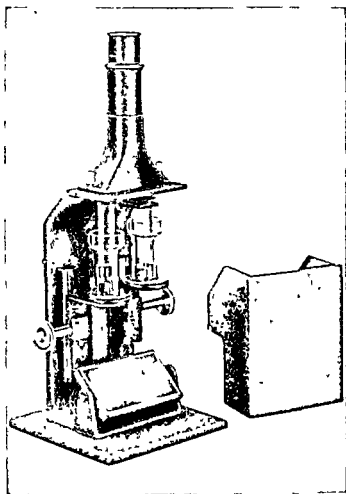
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W. H. DANIELSON, PH.D., CLEVELAND, OHIO

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IT IS readily appreciated that migraine ranks high among chronic ailments which result in loss of time and broken engagements for work and pleasure. Although migraine is not in itself a fatal malady, it makes its claim for attention by the periodicity and unpleasantness of the attacks. Accordingly, many attempts have been made through the ages to affect a remedy for this disorder and to discover its possible causative factor (Riley<sup>1</sup>).

The first direct evidence associating migraine attacks with possible changes in the acid-base balance of the blood was that presented by R. and S. Weismann Netter,<sup>2</sup> who determined the blood carbon dioxide content and pH in such cases. These authors found in eleven patients between attacks that the mean alkaline reserve was 61.6 volumes per cent and the mean pH 7.39, with limits of variation within the normal range. Bloods which were drawn during the crisis were found to be essentially identical with those drawn between attacks. These authors were able, however, to carry out a series of determinations in two cases where the attacks occurred at regular intervals. From the observations made upon these two cases the opinion was expressed that in certain cases of migraine the attacks are related to an uncompensated alkalosis which appears several hours before the attack develops. It was also reported that hyperventilation may result in an attack of migraine, probably through an excessive loss of carbon dioxide. Aside from these direct observations, the chief evidence of a possible disturbance of the acid-base balance of the blood lies indirectly in the reported beneficial effects of

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TABLE 1  
THE ACID-BASE BALANCE OF THE SPTM IN MIGRAINE

CASE	DATE	pH	BHCO <sub>3</sub>		BCL		BPR		TOTAL BASE		ICTEPUS INDEX	IF MAPKS
			M Eq	M Eq	M Eq	M Eq	M Eq	M Eq	MINUS TOTAL MEASURED ACID	TOTAL CHOLESTEROL MG		
1	10/31/41	7.40	26.6	105.2	16.1	156.4	8.5	171	At height of attack			
	11/ 4/31	7.43	25.6	103.2	16.8			210	Shortly following height of attack			
2	11/ 3/31	7.39	27.3	103.1	16.2	150.7	4.1	215	At height of attack			
	4/19/32	7.45	27.3	101.9		151.0		171	Normal			
	5/27/33	7.42	25.1	101.4	17.5	150.2	6.2	187	Shortly following height of attack			
	6/19/33	7.43	24.5	101.9	16.5	154.0	11.0		At height of attack			
	2/11/35	7.45	25.3	99.4	16.5	150.0	8.8		At first indication of headache, severe attack followed			
3		7.39	29.6	99.5	17.3			150	Four days following attack			
4	1	7.49	27.1	93.5	18.2	147.5	6.3	19	Shortly following height of attack			
		7.42	28.0	101.9	16.4			96	Normal			
		7.40	28.9	102	14.8	158.1	12.1	145	Shortly following height of attack			
	1	7.45	26.9	99	15.1	154.8	1.5		At height of attack			
5	1	7.41	27.2	101.0	17.2	147.9	5	148	Normal			
		7.41	24.0	10.7	16.5	144.6	0.4	157	At height of attack			
		7.43	26.7	10.4	17.8	150	5.4	117	Normal			
6	1	7.39	26.7	102.4	17.7			166	Normal			
	1	7.35	25.4	105.2	14.8	150.2	4.5	155	At height of attack			
		7.43	27.1	101.4	16.5	150.3	5.3	115	Normal			
7		7.40	24.6	100	16.0	149.8	8.0	100	Normal			
		7.42	24.0	98.2	17.3	146	6.8	182	Shortly following height of attack			
		7.45	26.1	105.0	16.4	153.7	6.2	152	At height of attack			
8		7.46	24.1	102.9	18.3	150.2	4.9		Normal			
9		7.41	27.6	101.0	15.6	150.2	8.1		Several hours before onset of attack			
10	1	7.40	27.6	102.0	16.0	150.0	6.0	152	Normal			
11		7.45	30.0	105.7	17.7			223	Morning following attack			
12		7.33	27.3	102.9	16.8			257	At height of attack			
		7.43	25.0	102.3	18.0	147.7	2.4	196	Normal			
*13		7.40	25.4	98.0	17.8	156.7	17.5		Morning following attack			
		7.46	26.9	99.6	17.9	146.6	2.2		Shortly following height of attack			
14		7.46	27.1	102.8	15.9	150.0	4.2		Shortly following height of attack			

\*Cases 3 and 13 were males

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## PRECIPITINS FOR STREPTOCOCCUS HEMOLYTICUS IN RHEUMATOID ARTHRITIS SERUMS\*

MARGARET STRAUB NEIL, AND EDWARD F. HARTUNG, M.D., NEW YORK, N. Y.

THE presence of agglutinins for hemolytic streptococci in high titers in the serums of patients with rheumatoid arthritis has been verified by most investigators of this problem.<sup>1-9</sup> Agglutinins for these organisms are not found at all or only in low titers in most serums from patients with osteoarthritis or from normal subjects. These findings, summarized in Table I, stand out as almost the only undisputed observations among the various bacteriologic and serologic studies on arthritis published in the last thirty six years. The results of the agglutination reactions have led many investigators to assume that hemolytic streptococci are involved, primarily or secondarily, in the etiology of rheumatoid arthritis. Certainly agglutination is not specific for any "typical strain" of streptococcus. Comparable results are obtained with various strains of *Streptococcus hemolyticus*.

The purpose of this study was to determine the presence of precipitins for various fractions of hemolytic streptococci in the serums of arthritis patients and to compare the results thus obtained with the agglutinin content of the same serum. It has been found that in some bacteria, such as in the pneumococcus, type specificity may be determined by a characteristic carbohydrate and group specificity by the protein fraction of the organism. Chemical determinants of type and group specificity have been demonstrated for the *Streptococcus hemolyticus* as well. Other workers have found in streptococci a type specific protein which permits distinction of types in agreement with the results of agglutination and mouse protection tests, and nucleoprotein which gives cross precipitin and complement fixation reactions with analogous fractions of other gram positive cocci, such as *Streptococcus viridans*, pneumococcus and staphylococcus. A group specific carbohydrate has been found which is characteristic of strains of human origin. Other fractions have been shown to be present but they are not important in our present study. It appeared possible that the precipitin tests might, perhaps, give more uniform and specific results with arthritis serum than those obtained by the usual agglutination tests first because the antigen might be prepared so as to include a greater concentration of the type specific and group specific substances, and second, because the antigen used in the precipitin tests might be less subject to variation.

Few reports on precipitins for hemolytic streptococci in relation to arthritis have been published. Seegal, Heidelberger, Jost, and Lyttle<sup>10</sup> performed pre-

\*From the Arthritis Clinic and the Department of Pathology and Bacteriology, New York Post Graduate Medical School and Hospital, Columbia University.

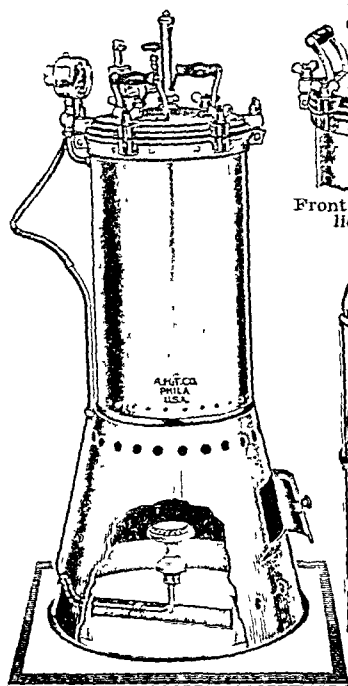
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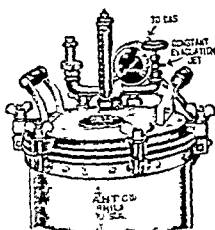
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trated by means of alcoholic precipitation. The amount of carbohydrate, the group specific substance contained in the crude HCl extract was less than 0.1 per cent as shown by chemical tests described below. To obtain appreciable amounts of carbohydrate this extract must be further concentrated.

The method of preparing the antigens is briefly as follows. A liter of plain broth of pH 7.6 was inoculated with 6 c.c. of a freshly growing broth culture of *Streptococcus hemolyticus*. After eighteen hours' incubation at 37° C., the sediment obtained by centrifugation was suspended in 0.85 per cent salt solution. To this was added sufficient normal hydrochloric acid to make a final concentration of N/20. This suspension was immersed in boiling water for fifteen minutes, cooled to room temperature and centrifugalized for thirty minutes. The supernatant liquid was neutralized with sodium hydroxide. The precipitate which formed was discarded after centrifugation and the resulting supernatant fluid was the crude antigen ready for use. About 15 c.c. of crystal clear, slightly yellowish antigen was obtained from each liter of broth culture.

In order to extract the type specific fraction, the crude hydrochloric acid extract was treated with three times its volume of 95 per cent alcohol and sodium acetate in proportion of 10 gm. per liter of extract. This mixture was allowed to stand in the refrigerator overnight. The precipitated substance was redissolved in 0.85 per cent saline. This process was repeated three times. The final solution which was used as the antigen contained protein M, the type specific substance, and less than 0.1 per cent of carbohydrate.

The following technique was used in performing the precipitin test. Three tubes were set up containing equal amounts of patient's serum, and varying amounts of antigen and saline, so that the total volume in each tube was 0.5 c.c. The fourth tube which was set up as a control contained only patient's serum and saline. Thus the first tube contained 0.4 c.c. of antigen, and 0.1 c.c. of serum, the second, 0.3 c.c. of antigen, 0.1 c.c. of serum, and 0.1 c.c. of saline, the third, 0.1 c.c. of antigen, 0.1 c.c. of serum, and 0.3 c.c. of saline. The control tube contained only 0.1 c.c. of serum and 0.4 c.c. of saline. The tubes were well shaken, placed in a water bath at 37° C. for two hours and at the end of this time a preliminary reading was made. The tubes were then placed in the refrigerator overnight and a final reading was made in the morning. The tests were not centrifugalized before reading.

#### RESULTS

In Table II it will be seen that the serum of 36 per cent of rheumatoid arthritis patients agglutinated strain AB<sub>11</sub> in serum dilution 1/160 and above. Strains NY<sub>1</sub> and C<sub>1</sub> gave results of 30 per cent and 24 per cent, respectively. Other hemolytic organisms obtained from the blood of septicemia subjects, and those isolated from the throat and stool gave a much lower percentage of positive results. RB<sub>1</sub>, a *Streptococcus viridans*, was agglutinated in 16 per cent of the serums, other *viridans* strains gave positive results in very small percentages. The serums from patients with osteoarthritis showed no agglutinins for any of the hemolytic organisms, and only 6 per cent showed agglutination for strain RB<sub>1</sub>. Agglutination tests with other green streptococci were negative. In the

ketogenic diets in the control of the migraine attacks (Schnabel,<sup>3</sup> Barborka,<sup>4</sup> Pollock and Barborka,<sup>5</sup> and Barborka<sup>6</sup>). That migraine may be associated with an alkalosis does not seem unreasonable, since it is a common observation that the uncompensated alkalosis following excessive bicarbonate administration may be accompanied by severe headache (Gatewood, Gaebler, Muntwyler and Myers<sup>7</sup>).

As a result of the uncertainty of the changes of the acid-base balance of the serum in migraine, an investigation was begun in 1931 with the object of making a comprehensive study of a few typical migraines over a considerable period of time. The plan was to obtain blood specimens at various intervals between and during the periods of attacks. Due to the lack of cooperation on the part of the patients this program was soon abandoned and the study resolved itself into obtaining blood from a number of patients at various periods in relation to the attacks.

The patients with migraine selected for the present study all presented severe paroxysmal headaches associated with various aura and accompanied by nausea, vomiting, visual disturbances and prostration. The acid-base balance procedures and the manner of handling the blood specimen were performed as previously described (Muntwyler and others<sup>8</sup>).

#### RESULTS AND DISCUSSION

The results of the acid-base balance studies of the serum in fourteen cases of migraine are collected in Table I. During the course of this study, it was possible to obtain two blood specimens at the first indication that an attack was coming on, eight at the height of the attack, nine shortly following the height of the attack, and eleven in between the attacks. It is apparent from a perusal of the data presented that there is no change in the acid-base balance from normal in migraine. This is also borne out when the average values for any of the periods in relation to height of the migraine attack are considered. Reviewing the acid-base values as a whole, the pH ranged from 7.33 to 7.49 with 27 of the 30 determinations falling between pH 7.39 and 7.46. The value of pH 7.49 in Case 4 may in all probability be explained as secondary to the extreme vomiting which occurred in this instance. This factor undoubtedly accounts for the only lowered serum chloride value (93.5 m.eq.) encountered in this series. All of the serum bicarbonate values remained within the normal range and the great majority of the figures were within the range 24.0 and 29.0 m.eq.

These observations substantiate the findings of R. and S. Weismann-Netter<sup>2</sup> who observed no change in the pH and carbon dioxide content of the blood either during or in between the migraine attacks. We were not able to study cases in which the migraine attack occurred at regular intervals as was the case with the above workers so that the acid-base balance studies could not be made as much as forty-eight hours before the attack. It is at such a period that the above workers found an uncompensated alkalosis. In Cases 2 and 9, however, the blood was obtained a number of hours before the onset of severe headache and accompanying symptoms. In these two instances there is also no evidence of a disturbance in the acid-base balance. It is rather difficult to understand how a supposed

extract were found, they were present, with the exception of one serum, for the other extracts as well and in comparable titers.

The type specific fraction M, obtained from the crude hydrochloric acid extract by alcohol precipitation, was used as an antigen in a subsequent series of tests. It was found that the results quite closely paralleled the agglutinin

TABLE III

AGGLUTINATION AND PRECIPITATION REACTIONS USING NY<sub>2</sub> ANTIGEN AND HCl EXTRACT

PA TIENT	AGE	DURATION OF DISEASE	TITER OF AGGLU- TININS	PRECIPI TINS	ANTHENT	AGL.	DURATION OF DISFAST	TITER AGGLU TININS	PRECIPI TINS
<i>I Rheumatoid Arthritis, 55 Patients</i>									
Hu	57	5 yr	1 1280	+++	Pe	25	3 mo	0	+++
Do	48	14 yr	1 1280	+++	Pr	26	3 yr	0	+++
Sl	54	7 yr	1 1280	+++	Pe	18	34 mo	0	+++
Co	54	2 yr	1 640	+++	Fl	33	5 yr	0	+++
Bl	48	8 yr	1 640	+++	Wa	28	2 yr.	0	+++
Mc	56	14 yr	1 640	+++	Nr	46	15 yr	0	+++
St	24	12 yr	1 640	+++	Br	58	6 wk	0	+++
Co	48	6 yr	1 640	+++	Ke	38	14 yr.	0	+++
Kn	52	1 yr	1 640	+++	Sm	49	6 mo	0	+++
Mu	54	4 yr	1 640	+++	Pe	30	3 yr	0	+++
Bo	60	12 yr	1 320	+++	Fe	50	1 yr	0	+++
Kn	45	14 yr	1 320	+++	De	33		0	+++
St	51	6 yr	1 320	+++	Li	22	4 yr	0	+++
Ba	55	1 yr	1 320	+++	Ma	41	5 yr.	0	+++
Br	58	8 yr	1 320	+++	Se	39	6 mo	0	+++
Ze	50	3 yr	1 320	+++	Hi			0	+++
Ja	28	2 yr.	1 320	+++	Ne	32	4 mo	0	+++
Ho	30	4 mo	1 320	+++	Ro	33	5 wk	0	+++
Ar	23	9 yr	1 320	+++	Se	47	3 mo	0	+++
Sc	47	3 yr	1 320	+++	Ha	49	14 yr	0	+++
Br			1 320	+++	Ma	38	14 yr	0	+++
Mc	42	8 yr	1 160	+++	Pe	18	3 yr	0	+++
Be	55	1 yr	1 160	+++	Hy	55	14 yr	0	+++
Go	46	2 yr	1 160	+++	Wi	54	5 mo	0	+++
To	47	7 mo	1 80	+++	Br	21	1 wk	0	+++
Mc	71	2 mo	1 80	+++	So	27	4 mo	0	+++
Mo	34	10 mo	1 20	+++	Se	38	4 yr	0	+++
Ke	33		1 20	+++					+++

*II Osteoarthritis, 20 Patients*

Bu			0	+++	Ro			0	+++
Ka			0	+++	Se			0	+++
Mc			0	+++	Ga			0	+++
St			0	+++	Mo			0	+++
Ko			0	+++	Co			0	+++
Dr			0	+++	Co			0	+++
Fr			0	+++	Be			0	+++
Re			0	+++	Ga			0	+++
Di			0	+++	Go			0	+++
Ba			0	+++	We			0	+++

*III Normal Subjects, 22 Patients*

1			1 20	+++	12			0	+++
2			0	+++	13			0	+++
3			0	+++	14			0	+++
4			0	+++	15			0	+++
5			0	+++	16			0	+++
6			0	+++	17			0	+++
7			0	+++	18			0	+++
8			0	+++	19			0	+++
9			0	+++	20			0	+++
10			0	+++	21			0	+++
11			0	+++	22			0	+++

uncompensated alkalosis forty-eight hours prior to the attack could be a contributing factor to migraine and then suddenly disappear so that entirely normal acid-base balance findings are observed when the migraine attack actually becomes manifest.

In addition to the acid-base balance determinations, estimations of cholesterol and icteric index were made in most of the cases. The cholesterol was determined on twelve of the fourteen cases. If the range of 140 to 170 mg. per 100 c.c. of serum is taken as normal (Myers and Wardell method<sup>9</sup>), then seven of the twelve cases had cholesterol values in excess of the upper normal; the highest value being 319 mg. per 100 c.c. The slightly elevated cholesterol value in this condition appears to have been the observation of several other workers (Moehlig,<sup>10</sup> and McClure and Huntsinger<sup>11</sup>). The icteric index was determined in thirteen of the fourteen cases. If the upper normal for this determination is taken as 6 (Bernheim<sup>12</sup>), then values greater than normal were found in ten of the cases; the highest value being 21. Both in the case of the cholesterol and icteric index there appeared no relationship between the concentration level and the presence or absence of the migraine attack.

#### CONCLUSIONS

1. There is no change of the acid-base balance of the serum either during or in between migraine headaches.
2. The blood cholesterol and icteric index are both frequently found slightly elevated in migraine, although the levels appear to hold no relation to the presence or absence of attacks.

#### REFERENCES

1. Riley, H. A.: *Migraine*, Bull. Neurol. Inst. New York 2: 429, 1932.
2. Weismann-Netter, R., and Weismann-Netter, S.: *Equilibre acide-base et migraine*, Compt. rend. Soc. de biol. 92: 341, 1925.
3. Schnabel, T. G.: *Experience With Ketogenic Dietary in Migraine*, Ann. Int. Med. 2: 341, 1928.
4. Barborka, C. J.: *Ketogenic Diet Treatment of Epilepsy in Adults*, J. A. M. A. 91: 73, 1928.
5. Pollock, L. W., and Barborka, C. J.: *Abdominal Migraine*, M. Clin. North America 11: 1665, 1923.
6. Barborka, C. J.: *The Ketogenic Diet and Its Use*, J. Am. Dietet. A. 8: 471, 1933.
7. Gatewood, W. E., Gaebler, O. H., Muntwyler, E., and Myers, V. C.: *Alkalosis in Patients With Peptic Ulcer*, Arch. Int. Med. 42: 79, 1928.
8. Muntwyler, E., Way, C. T., and Binns, D.: *The Acid-Base Equilibrium in Pathological Conditions. II. Alkalosis Observed in Hypertensive States*, J. Clin. Investigation 10: 489, 1931.
9. Myers, V. C., and Wardell, E. L.: *The Colorimetric Estimation of Cholesterol in Blood, With a Note on the Estimation on Coprosterol in Feces*, J. Biol. Chem. 18: 147, 1918.
10. Moehlig, R. C.: *Migraine: A Study Based on 100 Cases*, Endocrinology 15: 11, 1931.
11. McClure, C. W., and Huntsinger, M. E.: *Studies in Fat Metabolism. II. The Character of Blood Lipids in Hepatic Disorders, Including Migraine*, Arch. Int. Med. 43: 715, 1929.
12. Bernheim, A. R.: *The Icterus Index (A Quantitative Estimation of Bilirubinemia)*, J. A. M. A. 82: 291, 1924.

titer and the precipitin content when the crude hydrochloric extract was used. These findings are shown in Table V. Almost identical results were obtained, therefore, with the crude extract and with the type specific fraction itself.

TABLE V

COMPARISON OF AGGLUTININ AND PRECIPITIN TITERS USING NY, AS A WHOLE ANTIGEN AND THE CRUDE AND PURIFIED HCL EXTRACT

PATIENT	TITER OF AGGLUTININS	PRECIPITINS FOR HCL EXTRACT	PRECIPITINS FOR ALC EXTRACT	PATIENT	TITER OF AGGLUTININS	PRECIPITINS FOR HCL EXTRACT	PRECIPITINS FOR ALC EXTRACT
<i>I Rheumatoid Arthritis, 47 Cases</i>							
Di	1 1280	+++	+++	Pe	0	+++	+++
Co	1 640	+++	+++	Peh	0	+++	+++
Cor	1 640	+++	+++	Fl	0	+++	+++
Bl	1 640	+++	+++	Wt	0	+++	+++
Kn	1 640	+++	+++	Nt	0	++	+++
Mu	1 640	+++	+++	Sc	0	---	---
St	1 640	+++	+++	Sc	0	---	---
Br	1 320	+++	+++	Br	0	---	---
Ja	1 320	+++	+++	Nt	0	---	---
Ze	1 320	+++	+++	Ma	0	---	---
Bo	1 320	+++	+++	Sc	0	---	---
Kn	1 320	+++	+++	Ne	0	---	---
St	1 320	+++	+++	Br	0	---	---
Bo	1 320	+++	+++	Sc	0	---	---
Br	1 320	+++	+++	Pe	0	---	---
Sc	1 320	+++	+++	Fl	0	---	---
Wo	1 160	+++	+++	De	0	---	---
Be	1 160	+++	+++	Li	0	---	---
Go	1 160	+++	+++	Ro	0	---	---
Mc	1 80	+++	+++	Sc	0	---	---
To	1 80	---	---	Ha	0	---	---
Mo	1 20	---	---	Ma	0	---	---
Kc	1 20	---	---	Pe	0	---	---
<i>II Osteoarthritis, 20 Cases</i>							
Bu	0	+++	+++	Fr	0	---	---
Ka	0	+++	+++	Be	0	---	---
Pa	0	---	---	Re	0	---	---
Ga	0	---	---	Ga	0	---	---
St	0	---	---	Da	0	---	---
Mo	0	---	---	Go	0	---	---
Ko	0	---	---	Ne	0	---	---
Co	0	---	---	Mc	0	---	---
Dr	0	---	---	Ro	0	---	---
Co	0	---	---	Sc	0	---	---

## DISCUSSION

As has already been pointed out, our results in determining the agglutinin content in the serums of patients with arthritis are, in general, in agreement with those of other investigators. However, the percentage of serums which showed agglutinins in serum dilution of 1 160 and above are lower than those published by most workers (see Tables I and II). Close analysis of our work tends to show why this is so. Our objective when this research was undertaken was to test the usefulness and the accuracy of the agglutination test as an aid in differentiating between, and diagnosing rheumatoid and other forms of arthritis. The cases were selected with great care to distinguish between rheumatoid arthritis and other joint diseases but without regard to many factors which

cipitin tests on the serums from thirty-six cases of rheumatoid arthritis. They found strong precipitin reactions against *Streptococcus hemolyticus* protein fractions, whereas in the control groups precipitation was minimal or absent. Dawson, Olmstead and Jost<sup>11</sup> studied the precipitin content of the blood in patients with rheumatoid arthritis, using two nucleoprotein fractions designated D and K by Heidelberger, and a carbohydrate fraction C as antigens. They found that these chemical fractions of *Streptococcus hemolyticus* gave fairly similar reac-

TABLE I

A SUMMARY OF THE LITERATURE CONCERNING THE AGGLUTININ CONTENT OF THE SERUMS IN RHEUMATOID ARTHRITIS PATIENTS

AUTHOR	NUMBER OF PATIENTS TESTED	TITER CONSIDERED SIGNIFICANT	PER CENT OF SERUMS WITH SIGNIFICANT TITERS
Cecil, R. L., Nicholls, E. E., and Stainsby, W. J.	103	1:640 or higher	94.0
Nicholls, E. E., and Stainsby, W. J.	110	1:640	93.6
Nicholls, E. E., and Stainsby, W. J.	613	1:320	"High per cent"
Dawson, M. H., Olmstead, M., and Boots, R. H.	66	Extraordinary high titer	"Majority of cases"
Dawson, M. H., Olmstead, M., and Boots, R. H.	206	1:160 or higher	66.5
Keefer, C. S., Myers, W. K., and Oppel, T. W.	22	1:40 and 1:80	54.5
Blair, J. E., and Hallman, F. A.	62	1:160	85.0
Cox, K. E., and Hill, D. F.	28	1:320	90.0
McEwen, C., Bunim, J. J., and Alexander, R. C.	36	1:20 and higher	86.0

tions and that these results roughly paralleled the agglutination tests. Mc Ewen, Bunim, and Alexander<sup>9</sup> reported their findings of precipitins in rheumatoid arthritis serums, using a group-specific carbohydrate. They stated that 77 per cent of the serums from patients with rheumatoid arthritis and 24 per cent of those from normal subjects gave positive reactions.

#### MATERIAL

Only human strains of *Streptococcus hemolyticus* were used in the present precipitin studies. The results obtained with an antigen prepared from NY<sub>5</sub>, isolated by Dochez from the throat of a patient with scarlet fever were compared in part with those of extracts from two other hemolytic streptococci, K, an organism isolated from the throat of a patient with acute rheumatic fever, and M, isolated from a case of mastoiditis. The serums tested were taken from typical rheumatoid arthritis and osteoarthritis patients at the New York Post-Graduate Hospital. The tests were controlled with serums from normal subjects.

#### METHOD

Hydrochloric acid extracts of hemolytic streptococci were used as antigens. These were prepared, as described by Lancefield,<sup>12</sup> according to a modification of Porge's method. Our object was to extract from each organism both the carbohydrate and protein components. The type-specific protein fraction designated as M was then extracted from the crude hydrochloric acid extract and concen-

2 Agglutinins and precipitins are demonstrable in comparable titers

3 The precipitin reactions using the hydrochloric acid extract of hemolytic streptococci and the type specific fraction, which is precipitated out from this crude extract give results which closely parallel each other

4 The precipitin test is found to be less subject to variation, requires less time to perform, and the antigen may be kept more uniform over a longer period of time

#### REFERENCES

- 1 Cecil, R L, Nicholls, E E, and Stunsby, W J The Etiology of Rheumatoid Arthritis Am J M Sc 181 12, 1931
- 2 Nicholls, E E, and Stunsby, W J Streptococcal Agglutinins in Chronic Infectious Arthritis, J Clin Investigation 10 323, 1931
- 3 Nicholls, E E, and Stunsby, W J Further Studies on the Agglutination Reaction in Chronic Arthritis, J Clin Investigation 12 505, 1933
- 4 Dawson, M H, Olmstead, M, and Boots, R H Studies on the Etiology of Rheumatoid Arthritis II Agglutination Reactions with Hemolytic Streptococci in Rheumatoid Arthritis, Proc Soc Exper Biol & Med 28 421, 1931
- 5 Dawson, M H, Olmstead, M, and Boots, R H Agglutination Reactions in Rheumatoid Arthritis I Agglutination Reactions With *Streptococcus hemolyticus*, J Immunol 23 187, 1932
- 6 Keifer, C S, Myers, W K, and Oppel, T W Streptococcal Agglutinins in Patients With Rheumatoid (Atrophic) Arthritis and Acute Rheumatic Fever, J Clin Investigation 12 267, 1933
- 7 Blair, J. E, and Hullman, F A Streptococcal Agglutinins and Anti Streptolysins in Rheumatoid (Atrophic) Arthritis, J Clin Investigation 14 505, 1935
- 8 Cox, K E, and Hill, D F Chronic Arthritis, Serologic and Clinical Studies, Arch Int Med 54 27, 1934
- 9 Mc Ewen, C, Bunim, J I, and Alexander, R C Bacteriologic and Immunologic Studies in Arthritis II Results of Various Immunologic Tests in Different Forms of Arthritis, J LAB & Clin Med 21 465, 1936
- 10 Scogal, D, Heidelberger, M, Jost, E L, and Lyttle, J D Precipitins Against Fractions of Streptococci in Hemolytic Streptococcus Disease, Glomerular Nephritis Rheumatoid Arthritis, Proc Soc Exper Biol & Med 30 582, 1933
- 11 Dawson, M H, Olmstead, M, and Jost, E L Agglutination Reactions in Rheumatoid Arthritis, Comparison of Agglutinins and Precipitins for *Streptococcus hemolyticus* in Rheumatoid Arthritis Sera, J Immunol 27 355, 1934
- 12 Lancefield, R C The Antigenic Complex of *Streptococcus hemolyticus*, J Exper Med 47 91, 1928

TABLE II

AGGLUTINATION REACTIONS IN RHEUMATOID ARTHRITIS, OSTEOARTHRITIS, AND NORMAL SUBJECTS

NUMBER OF PA- TIENTS	BACTERIAL ANTIGEN USED	RHEU- MA- TOID ARTHRI- TIS	OSTEO- ARTHRI- TIS	NORMAL	RHEU- MA- TOID ARTHRI- TIS	OSTEO- ARTHRI- TIS	NORMAL
		PER CENT NEGATIVE IN ALL DILUTIONS			PER CENT POSITIVE IN DI- LUTIONS 1:160 AND ABOVE		
50	AB <sub>13</sub> Alpha prime streptococcus*	54	100	84	36	0	10
33	NY <sub>5</sub> <i>Streptococcus hemolyticus</i> †	60	89	-	30	0	-
33	C <sub>17</sub> <i>Streptococcus hemolyticus</i> ‡	60	89	-	24	0	-
50	Adamno <i>Strep. hemo. septicemia</i>	54	98	94	14	0	0
33	Throat M <i>Strep. hemo.</i>	69	96	-	6	0	-
33	Stool S alpha streptococcus	75	81	-	6	0	-
50	RB <sub>5</sub> <i>Streptococcus viridans</i> §	64	62	60	16	6	16
50	Allen <i>Strep. vir. septicemia</i>	86	100	100	4	0	0
33	Clawson, <i>Strep. vir.</i>	81	90	-	0	0	-
33	Clawson, <i>Strep. vir.</i>	75	81	-	6	0	-

\*Isolated, according to R. L. Cecil and others, from the blood stream of a patient with rheumatoid arthritis.

†Isolated by Dr. A. Dochez from the throat of a patient with scarlet fever.

‡From the throat of a patient with rheumatic fever.

§Isolated by Cecil from the blood of a patient with acute rheumatic fever.

||Isolated by Dr. B. J. Clawson and others, from a patient with acute rheumatic fever.

||Isolated by Dr. B. J. Clawson and others, from a patient with "chronic arthritis."

normal group, agglutination occurred in 10 per cent of the serums when strain AB<sub>13</sub> was used. The results with other hemolytic organisms were negative. When *Streptococcus viridans* organisms were used, 16 per cent of the normal serums showed agglutination with strain RB<sub>5</sub> but not a single serum agglutinated any of the other strains. In general our findings of agglutinins for hemolytic streptococci in the serum of patients with arthritis are in agreement with the results of other workers.

Table III compares the agglutinin and the precipitin content of serums from another group of patients with rheumatoid arthritis and osteoarthritis, and from normal subjects, using a suspension of the whole organism NY<sub>5</sub> as an antigen for the agglutination test and the hydrochloric acid extract of the same organism for the precipitin test. In this rheumatoid arthritis group 24 patients, or 44 per cent, showed agglutinins in titers of 1:160 and above. All but two of these 24 serums showed precipitins in varying titers. Generally speaking, the higher the agglutinin titer the stronger was the precipitin reaction. The remaining 31 serums showed agglutinins in low titers or not at all and only seven of them showed precipitins. In the group of 20 serums from patients with osteoarthritis, none showed agglutinins and only two showed precipitins, these in very low titers. In the normal group neither agglutinins nor precipitins were found.

We were interested to see if hydrochloric acid extracts of hemolytic streptococci other than NY<sub>5</sub> would give comparable precipitin reactions. In Table IV the results are tabulated on 11 rheumatoid arthritis, 11 osteoarthritis, and 11 normal serums. The serums were all tested for agglutinins for NY<sub>5</sub> and precipitins for the hydrochloric extracts of NY<sub>5</sub>, K and M, all strains of *Streptococcus hemolyticus*. It is obvious from this table that when precipitins for one

and the flask thoroughly shaken. When the mixing is complete, 9 ml of methyl sulphate is added in several portions, shaking thoroughly between additions. After a final thorough shaking, the flask is warmed in a water bath for an hour. A slow stream of nitrogen should be led through the flask and condensed during the methylation.

When the flask has cooled, it is disconnected, and its contents are transferred to a stoppered flask. (A preliminary concentration under reduced pressure at this point may be necessary.) A moderate amount of ether is added, the mixture thoroughly shaken and set away overnight. After twelve or fifteen hours a heavy scum will have formed if the pH of the mixture is near 7.0. This is the crude acid. It is filtered off, pressed between filter papers, and dried in the air. The yield is about 3 gm. When dry, the crude acid is a tan powder.

The crude acid is purified by extraction with dry acetone. It is best extracted repeatedly with boiling acetone in a reflux condenser. Many portions of acetone should be used, each extract filtered hot, and all the filtrates united. The filtrates are uniformly light tan in color. The united filtrates are decolorized with animal charcoal, then concentrated until a slight turbidity appears. Three or four volumes of hot benzene are added to the hot acetone solution and the mixture allowed to cool gradually. A crystalline precipitate, consisting of colorless rhomboid plates, parallelograms and long flattened spines, soon appears. The yield may be increased somewhat by adding more benzene or by distilling off some of the acetone through a fractionating column. The crystals are recrystallized from a hot acetone benzene mixture, and they reappear as rhomboid plates of uniform size and shape.

The crystals melt at 204 or 205° C with decomposition and gas formation. There is considerable sublimation at the melting point, and the sublimate consists largely of long, thick needles which melt at 205° C. It was not possible to observe the formation of crystalline material melting at 154.5° C from the decomposition products of the original crystals, which would have corresponded to dimethoxyindole.<sup>2</sup>

The crystals are soluble in water. The solution gives a precipitate with phosphotungstic acid, gold chloride and mercuric chloride solutions. There is no reaction with p dimethylaminobenzaldehyde.

#### COMMENT

Although further study is required to establish the exact composition of the crystalline substance isolated from liver extract, all the observed properties and the method of isolation indicate that it is identical with the substance isolated by Raper from a tyrosinase tyrosine mixture. Accordingly, the red substance in liver extract is presumably identical with the "red substance" of Raper, namely the 5, 6 quinone of dihydroindole 2 carboxylic acid.

Whatever the influence of this substance on pernicious anemia may be, it can now be stated that liver extract contains the first member of a new series of alpha amino acids. Strictly speaking, this substance is an alpha imino acid like proline. However, it is unique in that it is an indole, or really an indoline, and that it is present in the unstable quinone form.

TABLE IV  
COMPARATIVE PRECIPITATION REACTIONS USING ICH, EXTRACTS OF THREE  
HEMOLYTIC STREPTOCOCCI

RHEUMATOID ARTHRITIS				OSTEOARTHRITIS				NORMAL SUBJECTS			
CASE	TITER OF AGGLUTININS FOR NY <sub>5</sub> *	PRECIPITINS FOR		CASE	TITER OF AGGLUTININS FOR NY <sub>5</sub>	PRECIPITINS FOR		CASE	TITER OF AGGLUTININS FOR NY <sub>5</sub>	PRECIPITINS FOR	
		NY <sub>5</sub>	K*			NY <sub>5</sub>	K			NY <sub>5</sub>	M
Bo	1:320	++	++	Bu	0	++	+	1	0	---	---
Be	1:160	++	++	Ka	0	+	+	2	0	---	---
Go	1:160	++	++	Mo	0	---	---	3	0	---	---
Mo	1:20	++	++	Da	0	---	---	4	0	---	---
Pr	0	++	+	Go	0	---	---	5	0	---	---
Sc	0	---	---	Ne	0	---	---	6	0	---	---
Po	0	---	---	Pa	0	---	---	7	0	---	---
De	0	---	---	Ga	0	---	---	8	0	---	---
Pe	0	---	---	Mc	0	---	---	9	0	---	---
So	0	---	---	Sc	0	---	---	10	0	---	---
Sc	0	---	---	Ko	0	---	---	11	0	---	---

\*NY<sub>5</sub>, Strain of *Streptococcus hemolyticus* isolated by Dr. Dochez from the throat of a patient with acute rheumatic fever.

K, Strain of *Streptococcus hemolyticus* isolated from throat of patient with acute rheumatic fever.

M, Strain of *Streptococcus hemolyticus* isolated from case of acute mastoiditis.

The reticulocyte response was fairly definite and quite characteristic in time of occurrence. The patient's blood count was 1,020,000 RBC with 28 per cent reticulocytes on the first day. On the second day the reticulocyte percentage was 30, on the third, 30, on the fourth, 53, on the fifth, 60, on the sixth, 48, and on the eighth, the reticulocytes had decreased again to 15 per cent. Obviously, the peak of the reticulocytic response was far below that predictable for this grade of anemia. Nevertheless, the characteristic time of the response, and the definite rise and fall of the number of reticulocytes parallel with the administration of the "red substance" strongly suggest that there had been a definite although rather weak stimulation of hematopoiesis.

## REFERENCES

- 1 Jacoby, H. R. On the Nature of the Antipernicious Anemia Principle. II Identification of the 5, 6 Quinone of Dihydroindole 2 Carboxylic Acid in Liver Extract, *J. Lab. & Clin. Med.* 22: 890, 1937.
- 2 Raper, H. S., and Speakman, H. B. The Tyrosinase Tyrosine Reaction. IV. Note on the Identity of Tyrosinase From Different Sources, *Biochem. J.* 20: 69, 1926.

## IS A LASTING ACTIVE IMMUNITY AGAINST DIPHTHERIA OBTAINABLE WITH A SINGLE INJECTION OF ALUM PRECIPITATED TOXOID?\*

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IT IS unnecessary to reiterate the obvious advantages of the single injection-method in the active immunization against diphtheria. This method was made possible in my own use of sterile lanolin as a menstruum,<sup>3</sup> but more recently by the popular alum precipitated toxoid.

The question has been raised as to the relative immunizing power of one dose of alum precipitated toxoid, and on a statistical basis with certain preparations in several different laboratories, the conclusion has been reached that the single injection of alum precipitated toxoid is not sufficient to provide a sufficiently lasting immunity.

Fitzgerald<sup>1</sup> finds a single dose of 1 c.c. of alum precipitated toxoid (20 Lf doses per c.c.) is decidedly inferior to 3 doses of the same material. He supports this statistical conclusion with the theoretical consideration that a second antigenic stimulus is required to secure the highest immunity response.

This theoretical point seems not to be well taken because Farago<sup>2</sup> has recently shown that there is a maximal antigenic quantity of the toxoid absorbed daily from the depot of the single injection for a period of at least two weeks, with only a slight lessening at three weeks. Farago demonstrated

\*From the Department of Pediatrics and the Department of Applied Immunology, Jewish Hospital.

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seem to play a part in determining the agglutinin titer. Had we been interested in finding out the time in the course of the disease when patients developed streptococcus agglutinins and how long these antibodies remain in the circulating blood after the disease process has become quiescent, our selection of cases would have been different. The age of the patient, the season of the year, the duration of the disease, whether of a few weeks or of many years, and the activity of the process were not taken into consideration. Only one serum was taken from each patient. Our figures, it should again be pointed out, include only those serums which showed a definite agglutination in serum dilution 1:160 and above, a fact which makes it impossible to compare our percentages of "positive" results with those of some workers.

Precipitin tests when using the crude hydrochloric acid extract and the concentrated extract which contained the type-specific fraction, gave results which were almost identical with each other and with the agglutination tests. The hydrochloric acid extract contained both the group-specific carbohydrate C and the type-specific protein M. The amount of C in this crude extract was less than 0.1 per cent and has been used as a source of purified C by Lancefield. Whether the concentration of C in this extract was sufficient to account for our precipitin results or whether our results when using this extract were due to M, or a combination of both fractions, cannot be answered at present.

The chemical behavior of these extracts is summarized in Table VI. From this it is seen that the HCl extract did not contain a sufficient concentration of protein to give a positive biuret or Millon reaction. Its carbohydrate content was

TABLE VI

TESTS FOR CARBOHYDRATE AND PROTEIN ON BOTH EXTRACTS USED AS ANTIGENS

	CRUDE HCl EXTRACT	PROTEIN M FRACTION
Biuret	Negative	Slightly positive
Millon	Negative	Negative
Molisch	Slightly positive	Slightly positive
Benedict	Negative	Negative

insufficient to give a positive Benedict test but did give a slightly positive Molisch reaction. The purified M extract gave a positive biuret reaction and a slightly positive Molisch reaction.

The hydrochloric acid extracts from the three organisms we used gave comparable precipitin results. This finding cannot be interpreted as meaning that these streptococci were of the same type. All three extracts supposedly had in common the group-specific carbohydrate C. It may be, however, that if we had extracted only pure type-specific M from each of these organisms, we would not have obtained identical results. It may be that only certain types of hemolytic streptococci are involved in the etiology of rheumatoid arthritis. Work is now in progress to extract M from hemolytic streptococci of many sources to test this hypothesis.

## CONCLUSIONS

1. A greater percentage of serums from patients with rheumatoid arthritis show agglutinins in high titers for hemolytic streptococci than do serums from patients with osteoarthritis or from normal subjects.

and evolves no gas in vivo or vitro. It has great adsorptive properties, and when mixed with feces in vitro, the color, odor, toxins, acid, and almost 100 per cent of the bacteria are removed, leaving a clear supernatant fluid.

The toxicity of this mixture, when administered per rectum in large quantities, was determined experimentally in animals by M. R. Thompson, Professor of Pharmacology of the University of Maryland. Six dogs and six cats were injected daily with this mixture for a period of thirty days. The dosage per day was predetermined for each animal by noting the volume of fluid necessary to fill the colon, not exceeding the amount which could be easily retained. As controls, six dogs and six cats similarly received rectal injections of tap water. The animals were weighed three times a week, and observed daily. The appearance of the stools was noted. These studies revealed no toxic effects upon the animals.

Following these experiments, the mixture was tried on patients convalescing from acute attacks of ulcerative colitis who continued to show blood streaked stools. The quantity of fluid to be administered rectally was determined with the aid of a fluoroscope in six patients. The average capacity of the "con-

TABLE I  
EFFECT OF MIXTURE UPON 26 ULCERATIVE COLITIS PATIENTS

NAME	SEX	AGE	DURATION OF ILLNESS (YEARS)	DIAGNOSIS*	NUMBER OF TREATMENTS	NUMBER OF RELAPSES	CLINICAL AND SIGMOIDOSCOPYIC RESULTS
H S	F	27	2	B D	26	2	Good
R P	F	22	15	B D	8	None	Good
M S	F	21	55	B D	10	None	Good
H S	M	34	15	S	6	None	Very good
A C	F	33	30	B D	10	None	Good
G S	M	33	10	B S	4	None	Very good
A S	F	37	80	B S	21	1	Good
G G	M	23	10	S	30	2	Fair
H M	F	30	130	B D	15	2	Good
M C	M	22	20	B D	8	None	Good
H H	M	38	40	B D	12	1	Good
B H	F	20	50	B D	10	None	Good
L M	M	28	40	B D	6	None	Very good
P W	M	18	40	?	18	2	Fair
S D	F	19	60	B D	9	None	Good
B H	F	23	30	B D	6	None	Very good
L S	M	16	15	P	6	None	Very good
R N	F	20	20	P	8	None	Good
M F	M	34	20	A & B	6	None	Very good
S R	M	44	40	S	4	None	Very good
R M	F	24	30	B D	12	1	Good
B F	F	73	150	B D	24	3	Poor (mental case)
F B	F	33	25	B S	9	None	Good
S K	F	20	40	A	8	None	Good
M O	M	22	20	B D	10	1	Good
S W	M	19	25	B D	10	None	Good

\*Code to abbreviations

B D Bacillary dysentery

A Amebic dysentery

A & B Amebic and bacillary dysentery

S Nonhemolytic streptococcus infection

B S Bacteroides dysenteriae infection

P Paratyphoid B bacillus infection

? Undetermined etiology

## ON THE NATURE OF THE ANTIPERNICIOUS ANEMIA PRINCIPLE II\*

### IDENTIFICATION OF THE 5, 6-QUINONE OF DIHYDROINDOLE-2-CARBOXYLIC ACID IN LIVER EXTRACT

HENRY R. JACOBS, M.D., CHICAGO, ILL.

THE fact that experiments designed to isolate the active principle of liver extract often end with the formation of large amounts of humin suggested that the active principle might be a "pre-melanin" or a "melanogen." Proceeding on this assumption, experiments were made to isolate it as a derivative. The fundamental work of Raper<sup>1</sup> was used as a guide.

*A. Similarity of Liver Extract to the "Red Substance" of Raper.*—The purest liver extracts of commerce uniformly have a red color. This color is discharged: (a) wholly by hydrogen peroxide, (b) partially by zinc and acetic acid, (c) partially by standing in vacuo. After a few days the decolorized solution deposits a fairly insoluble dark precipitate, (d) largely by standing in contact with sulphurous acid.

The red color is deepened by standing in contact with air in faintly acid solution. When dry liver extract is heated to about 180° C., it gives off large amounts of carbon dioxide and changes its properties completely, becoming then an acrid, insoluble dark brown mass.

*B. Isolation of Dimethoxyindole-Carboxylic Acid.*—The apparent instability of the active principle demands a mode of treatment quite like that used by Raper to identify the quinone of dihydroindole in the reaction mixture of tyrosinase and tyrosine.

One hundred grams of commercial liver extract powder (Lilly) is stirred up in 125 ml. of 0.1 N HCl. After stirring and shaking the mixture for about ten minutes, it is centrifugated and decanted. Sulphur dioxide gas is bubbled through the liquid to saturation. The liquid is then set aside for three or ten days, during which time a tan precipitate appears. The liquid is filtered, and the filtrate concentrated under reduced pressure until it becomes thick. (A slow stream of nitrogen bubbling through the liquid during evaporation prevents bumping.) In order to retard reoxidation at this point, 20 ml. of 0.1 N HCl saturated with SO<sub>2</sub> is added.

The liquid is then placed in a flask which can be attached to a reflux condenser equipped with a dropping funnel, inlet and outlet tubes for nitrogen (or hydrogen) gas, and a connection to a vacuum pump. The apparatus is now exhausted and refilled with nitrogen gas several times to remove all oxygen. Then 50 ml. of 20 per cent NaOH is introduced through the funnel,

\*From the Department of Medicine, University of Chicago.

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4 This therapy is indicated only in the convalescent patient whose stools continue to be streaked with blood

5 Coincidentally, the number of stools decrease from six or seven, to one or two in twenty-four hours

6 The mucous membrane of the rectosigmoid shows definite progressive improvement, and finally, healing, as observed sigmoidoscopically before, during, and after treatment

The author is indebted to John Wyeth and brother, of Philadelphia, for their technical assistance

955 EASTERN PARKWAY

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## CHORIOANGIOFIBROMA OF THE PLACENTA\*

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### REPORT OF A CASE

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B W RHAMA, M D , F A S C P , F O R T W A Y N E , I N D

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**A**MONG the curious anomalies of nature are tumors of the placenta, the common variety being the hydatid mole. Chorionepitheliomas, the malignant form, although rare are also met with in every pathologist's experience. Considerable speculation exists as to the etiology of tumors of the placenta, some even denying the possibility of placental tumors. Others believe syphilitic placentitis to be the etiologic factor, while others believe blood stasis from kinks, etc., produces angiomatous dilatations simulating newgrowths. Gradually, however, as evidence is forthcoming, it is being recognized that there is still another form of benign tumor of the placenta of unknown etiology in which there occurs capillary angiomata of the villi, with a richly cellular embryonic connective tissue, which have been called by Dienst "allontagenous myxofibrocappillary angiomata of the chorion." According to the literature, John Clarke in 1798 was the first to report a solid tumor of the placenta the size of an apple, and containing epithelium, blood vessels and connective tissue. R S Siddall of the Obstetric Department of Henry Ford Hospital (*Am J Obst & Gynec* 8:554, 1924), collected from the literature and records 131 cases of the extremely rare chorioangiofibroma of the placenta. Nothing unusual is observed in the maternal history in these cases, except an increased risk from hemorrhage. On the part of the fetus, due to hydramnios, and prematurity, a mortality of 37.6 per cent to 41.0 per cent has been observed.

Chorioangiofibromas are of variable size and consistency and so far as our present knowledge goes, are benign. These tumors range in size from a grain of wheat to the size of an apple. They are sharply circumscribed and may be single or multiple, as many as 4 to 6 separate growths have been described. Their capsule is made up of compressed villi, and are connected

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\*Reported at Methodist Hospital Staff Conference Sept 6 1935  
Received for publication October 6 1936

The effect on pernicious anemia, and the theoretical implications with respect to the formula of hematin and allied pigments, will be presented in a subsequent paper.

#### REFERENCES

1. Raper, H. S.: XIV. The Tyrosinase-Tyrosine Reaction. VI. Production From Tyrosine of 5:6-Dihydroxyindole and 5:6-Dihydroxyindole-2-carboxylic Acid—The Precursors of Melanin, *Biochem. J.* 21: 89, 1927.
2. Oxford, A. E., and Raper, H. S.: LXII. Synthesis of 5:6-Dimethoxyindole and Its 2-Carboxylic Acid, *J. Chem. Soc.* 1: 417, 1927.

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### ON THE NATURE OF THE ANTIPERNICIOUS ANEMIA PRINCIPLE III\*

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#### THE RESPONSE OF A CASE OF PERNICIOUS ANEMIA TO THE ORAL ADMINISTRATION OF A TYROSINASE-TYROSINE MIXTURE

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HENRY R. JACOBS, M.D., CHICAGO, ILL.

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THE 5:6-quinone of dihydroindole-2-carboxylic acid has been partially identified in liver extract.<sup>1</sup> Whether this substance is the active principle can be learned only through clinical trial. Because of its instability, it cannot readily be synthesized in pure form, and hence recourse must be made to the use of impure mixtures. Raper<sup>2</sup> showed that this substance was a product of the action of tyrosinase on tyrosine in the presence of oxygen. One mode of trial then is the administration of the red substance appearing in a tyrosinase-tyrosine mixture.

A suitable case of pernicious anemia presented itself before a purified preparation of tyrosinase could be made. However, the scarcity of suitable experimental cases required its use, if only in a preliminary fashion.

*Preparation of the Mixture Containing the Quinone.*—Following Raper's outline in general, the "red substance" was freshly prepared each day as follows: 100 c.c. of raw potato scrapings are diluted with 100 c.c. of phosphate buffer of pH 6.0. One gram of tyrosine is added to the mixture, and a stream of oxygen bubbled through for twenty or forty minutes. The mixture becomes lavender in color, which by centrifugation and decantation can be separated into a clear red liquid and a gray precipitate. The precipitate is again suspended in 100 c.c. of phosphate buffer of pH 6.0 and oxygenated for another half hour. After centrifugation, the supernatant liquid is decanted. The two portions of liquid represent the daily dose given to the patient.

The patient tolerated the solution well for four days. On the fifth day the patient had diarrhea and complained of nausea. However, during the first four days the red material from 4 gm. of tyrosine had been retained. The treatment was stopped because of the laxative effect of the solution.

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\*From the Department of Medicine, University of Chicago.  
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## CASE REPORT

Mrs R A, aged twenty-eight years, delivered Aug 1, 1934, of a normal baby girl, a normal pregnancy and delivery. Began menstruation again in November, 1934. Next pregnancy estimated to have taken place Jan 27, 1935, and date of confinement estimated to be Nov 4, 1935. On Sept 28, 1935, Dr Doster Buckner made a normal delivery of baby and placenta. It was apparently a full term gestation and a normal baby, and there had been nothing abnormal about the gestation. Several loculated nodules were, however, noted on the placenta, and it was sent to the laboratory for examination. This was



Fig 3—Low power. Junction between placenta and tumor. Note thin capsule and density of this tumor area.

a normal presentation, and there was no hydramnios nor hemorrhage. Urine showed no pathology, and syphilitic stigmas were absent.

## DESCRIPTION OF PLACENTA AND TUMOR

The placenta was irregularly ovoid in shape, with the cord inserted concentrically, 5.5 cm from the placental margin. After being hardened in formalin the placenta weighed 750 gm (25 oz), measured 18 by 14 cm across and 4 to 5 cm in thickness. About 2 cm from the cord were seen 8 irregular solid nodules easily enucleated and having the smoothness, color, and consistency of kidney substance. These nodules were covered by a more or less fibrous

## THE CONTROL OF RECTAL BLEEDING IN THE CONVALESCENT ULCERATIVE COLITIS PATIENT\*

WILLIAM Z. FRADKIN, M.D., BROOKLYN, N. Y.

LOCKHART-MUMMERY, in his book on *Diseases of the Colon and Rectum*, referring to the treatment of ulcerative colitis, states, "The best solution which I have yet tried is one which I have been using lately, and consists of bismuth subgallate (5 per cent) in suspension in olive oil." It is administered rectally. He also adds, "I have seen most spectacular results from this solution, the diarrhea being checked within twenty-four hours." The results obtained with the kaolin-aluminum hydroxide mixture and reported in this paper are equally as spectacular in many of the cases.

The continued presence of blood in the stools of a convalescent, ulcerative colitis patient, presents an important therapeutic problem. The severe degree of anemia found in most of these patients is mainly due to the chronic loss of blood with the frequent rectal discharges. All efforts to raise the hemoglobin are, therefore, doomed to failure unless the bleeding, as well as the number of bowel movements, is controlled.

On sigmoidoscopic examination, petechial hemorrhagic areas are noted which ooze blood on the slightest trauma. Small superficial ulcerations are also present which bleed readily during active peristalsis or defecation. Most of these lesions are in the distal end of the colon, and the stools passed are mixed with bright red blood.

In order to heal these lesions, antiseptic irrigations, astringent solutions, insufflations of bismuth and calomel, or suspensions of kaolin have been used and have given unsatisfactory results. A suspension of kaolin in aluminum hydroxide gel was then tried. This was given orally four and five times daily in tablespoonful doses. Although the results were better, the frequent administration of large doses of the mixture interfered with the digestion of the patient, and had to be discontinued.

The thought therefore came to mind of administering a similar mixture per rectum. This method would have the advantages of placing the medicament in immediate contact with the lesions, and eliminating the digestive disturbances. Mineral oil was incorporated in the mixture in order to avoid the formation of hard fecal concretions which might irritate the inflamed mucous membrane. The final mixture consisted of 20 per cent kaolin, 10 per cent mineral oil, and 70 per cent of a gel of aluminum hydroxide, equivalent to  $2\frac{1}{2}$  per cent of  $Al_2(OH)_6$ . The results of treatment with this mixture were strikingly beneficial. The bleeding stopped while the number of bowel movements decreased.

Purified kaolin, when suspended in aluminum hydroxide gel, is a white, viscous cream. It is odorless, tasteless, somewhat astringent, neutral in reaction,

\*From the Department of Gastroenterology, Jewish Hospital of Brooklyn.  
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## BLOOD LIPID STUDIES IN A CASE OF XANTHOMATOSIS ASSOCIATED WITH HEPATIC DAMAGE\*

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THE etiology of xanthomatosis is poorly understood, but it is generally recognized that this condition is associated primarily with a disturbance in lipid metabolism. Rowland<sup>1</sup> has pointed out that xanthomatosis may be associated with hepatic damage and an accompanying disturbance in lipid metabolism. It has been shown in experimental animals that the liver is the only organ in which the feeding of diets rich in cholesterol produces an increased concentration of cholesterol ester and neutral fat.<sup>2, 3</sup> Best and coworkers<sup>4</sup> have demonstrated that both choline and betaine would prevent fat deposition in the livers of normal animals fed diets with high fat or high cholesterol content. From clinical observations alone, Bloch<sup>5</sup> believes that the liver is the regulator of lipid metabolism and, therefore, that any disturbance in its function may be the cause of lipid imbalance.

The therapeutic procedures followed in xanthomatosis have been unsatisfactory principally because the pathologic physiology is not clear. Since the majority of cases of xanthomatosis have an hypercholesterolemia, cholesterol poor diets have been fed. The blood cholesterol has been reduced and the lesions have disappeared in some cases,<sup>6, 7</sup> but in others there has been no effect.<sup>8</sup> Thyroid extract has been recommended<sup>9</sup> on the basis of the lowered blood cholesterol values obtained in hyperthyroidism. Insulin has also been found to reduce hypercholesterolemia temporarily.<sup>10</sup> The use of other tissue extracts has not been reported.

In the present case of xanthomatosis following hepatic damage, neither the amount nor the ratio of the lipid constituents of the blood nor the clinical condition of the patient was appreciably changed after the ingestion of a "fat free" diet supplemented in succession by betaine, choline, thyroid extract, liver extract, and insulin.

### CLINICAL REPORT OF CASE

R E H, an American, male, shoemaker, aged forty, was admitted to the University of Virginia Hospital on May 14, 1934, complaining of painful nodules on the palmar surfaces of his hands and of similar lesions on his body. The patient had been essentially healthy until five years before admission, when he noted a penile lesion which his physician did not consider syphilitic. However, when a generalized body eruption occurred four months later, the physician began antisyphilitic therapy without waiting for the Wassermann report, which later proved to be negative. In spite of three additional negative tests, six doses of arsphenamine were administered intravenously. During the course of treatment the patient "turned yellow" with highly colored urine, light colored stools and itching, which was followed by an exfoliation of the skin of the entire body. At this time the patient was given twelve injections of sodium thiosulphate. The exfoliation gradually disappeared but the yellow discoloration

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valescent colon" was about 16 ounces. Since the lesions are found in the distal half of the colon, it was decided that a 10-ounce mixture would be ample for one treatment. When a patient passed three or more stools per twenty-four hours, the medication was administered without a preliminary saline irrigation. Otherwise, a small, low saline enema was given about two hours before taking the treatment, to permit the medication to come into immediate contact with the lesions. Six ounces of the mixture was diluted with four ounces of warm water. The treatments were carried out three times weekly, and then gradually reduced to once a week, as improvement was noted. The mixture was instilled slowly, taking fifteen to twenty minutes for the procedure. The first and second rectal injections were usually not retained for more than one-half hour. As the treatments progressed, the patients were able to retain the medication overnight for eight to ten hours. The first stool following a treatment was soft, or formed, and resembled the color of the medication. The blood streaks disappeared after the third or fourth week. In a few cases only four or six treatments were necessary. When blood streaking recurred, one or two weeks of treatments readily controlled it.

Twenty-six patients convalescing from acute attacks of ulcerative colitis were treated rectally in the manner outlined. There were fifteen bacillary dysentery infections, one amebic infection, one double infection with *E. histolytica* and dysentery bacilli, two paratyphoid infections, three Bagen diplostreptococci infections, and three infections with nonhemolytic streptococci. One case was of undetermined etiology. The diagnosis of bacillary dysentery was based on positive cultures or positive agglutination tests in high dilutions. These patients had been treated in the acute stages with intravenous medication, antidysenteric polyvalent serum, autogenous vaccines, or drugs, depending upon the bacteriologic findings. Because of the continued presence of specks and streaks of blood in their stools, associated with mild lesions found sigmoidoscopically, treatments with the above mixture were instituted.

The average number of treatments which were necessary to control the passage of blood-streaked stools was 11.4. The average number of weeks required for treatments was 7.7. Nine patients had a total of fifteen relapses during a period of nineteen months. Seventeen patients had no relapses during the same period. In most of the cases, treatments were continued for more than six weeks, although the bleeding was controlled within the third or fourth week. Sigmoidoscopic examinations were repeated as soon as the patients reported no visible blood in the stools. These observations in the majority of cases revealed clean, reddened, granular mucous membranes with only occasional superficial ulcerations which oozed slightly on manipulation. After further treatments, these lesions healed promptly leaving a pale, pink, granular mucosa.

#### CONCLUSIONS

1. This therapy is not a cure for ulcerative colitis.
2. It must not be used in the acute stages of the disease.
3. It must supplement or follow specific therapy whether in the form of drugs, serum, or vaccines.

continued. It was not until about two years before admission that he noticed an increasing number of yellowish nodules on his hands. Within the next few months, similar nodules appeared on other parts of the body.

Physical examination showed the patient to be fairly well developed and nourished. There was a definite icteric tinge of his sclerae and skin. Many firm yellowish nodules,

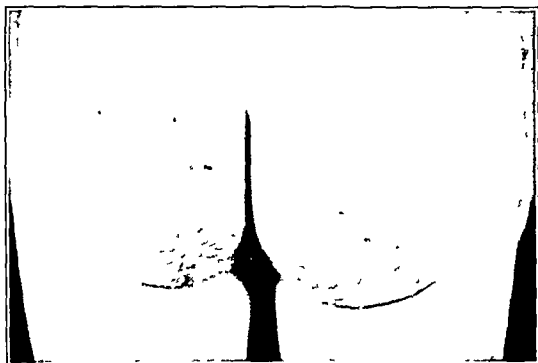


Fig. 3—Lesions on buttocks



Fig. 4—Lesions on testes

varying from 1 mm. to several centimeters in diameter, were visible. These xanthomas were most abundant on the hands, eyelids, elbows, knees, scrotum, and buttocks. A smooth tender liver edge was felt 12 cm. below the costal margin. The spleen was palpable and moderately enlarged. Additional abnormalities noted were a soft blowing systolic murmur at the apex of the heart, an easily reducible right inguinal hernia, and an absence of the biceps, patellar and ankle reflexes.

to the placenta by blood vessels. Clinically according to Curtis (*Obstetrics and Gynecology*, vol 1), they have little or no effect on the mother or on the course of pregnancy and labor except that in the presence of large tumors



Fig. 1—View of placenta at cord attachment



Fig. 2—Cross-section through largest tumor. Note multiple small tumors deep in placental substance

hydramnion, with its high fetal mortality, due to immature labor, is almost the rule, and during the third stage of labor there may be excessive hemorrhage as the result of deficient uterine contractions.

per cent) was obtained during the preliminary period and the concentration dropped to 907 mg per cent within a relatively short time. After the administration of betaine, the lowest value obtained was 632 mg per cent. It is believed that this low value represents a continued preliminary decrease due to the

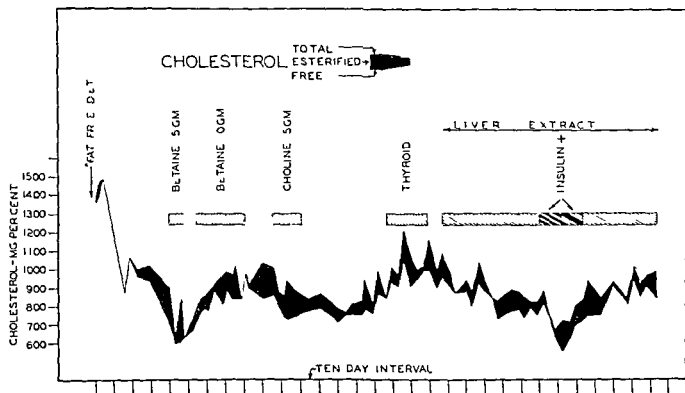


Fig 5—The effect of diet and medication on the cholesterol concentration in the blood plasma.

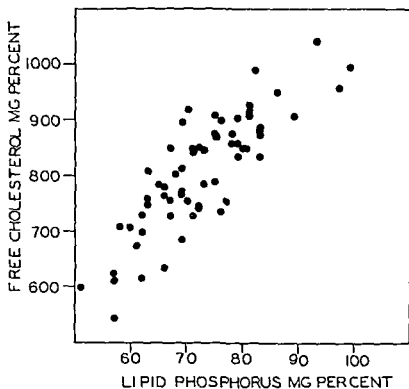


Fig 6—Relation between free cholesterol and phosphatid phosphorus

“fat free” diet, since further administration of betaine was accompanied by an increase in cholesterol concentration. The highest value obtained after the preliminary period was 1210 mg per cent when thyroid extract was given. The lowest value for the free cholesterol (545 mg per cent) was seen when the patient was receiving liver extract supplemented with insulin.

chorio-amniotic capsule containing numerous blood vessels and easily separated from the nodules. There were several large nodules; one measured 5 by 3.5 by 2.5 cm. and looked like a small kidney. Another was double, a smaller nodule growing out from the larger one. The under one measured 5.5 cm. across, and the two protruded out from the placenta 5 cm. The largest nodule is a hemisphere 4.5 cm. in diameter, including its capsule, pearly white to pink in color, pierced by cavernous blood vessels and extending through and protruding from both sides of the placenta. This round nodule is covered by a thick capsule of chorion and over the top of the nodule in the capsule runs a large blood vessel from the cord. Besides these 8 large protruding nodules there are numer-

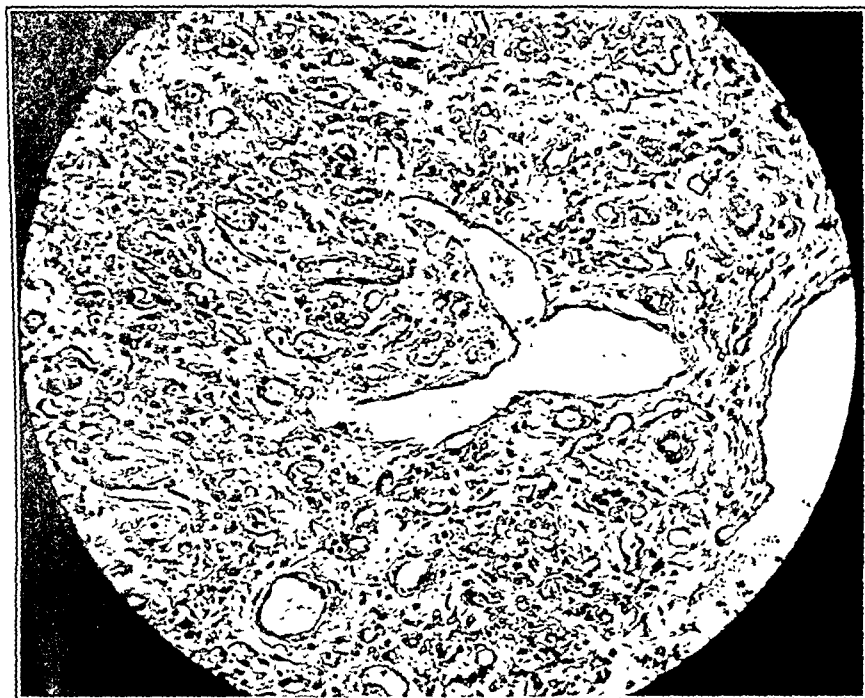


Fig. 4.—High power. Dark colored angiomatous area with many small and dilated capillaries.

ous small pinkish white nodules 0.5 to 1.5 cm. in diameter, more intimately associated with the placental substance, some of them being deep in the substance of the placenta.

#### MICROSCOPIC EXAMINATION

These tumors are separated from the placenta proper by a thick coat of compressed syncytium or Langhans' cells. The mass is composed of fibrous tissue in which are nests of capillaries of large and small diameter, lined with single layers of epithelium. Upon the relative proportions of cells to vessels in different masses depends the consistency and color which varies from pinkish white to dark brown. The connective tissue is loosely areolar and consists of spider connective tissue cells. In the pinkish white areas, the connective tissue stroma is quite dense.

*Phosphatid Phosphorus*—The values for phosphatid phosphorus varied between 51 and 99 mg per cent during the course of the observations. These amounts may be as much as ten times the normal values. The relationship of free cholesterol to phosphatid phosphorus is shown in Fig. 6. It can be seen that there is a good parallelism between these substances.

*Partition of the Total Plasma Lipids*—The lipid partition in 45 complete analyses is presented in Table I. The small percentages of cholesterol esters are in accordance with the findings of Epstein<sup>19</sup> who showed that cholesterol esters decreased in direct proportion to the severity of the damage in degenerating livers. The phosphatids comprised about 60 per cent of the total lipids. The percentages of free cholesterol and cholesterol esters and phosphatids remained fairly constant despite wide fluctuations in total lipids. It can be seen that the concentration of the total lipids was from four to six times the normal values.

*Analysis of Xanthoma*—One of the xanthomatous lesions, removed from the elbow and stripped of skin and connective tissue, weighed 0.625 gm. The following values on the basis of wet weight were obtained:

Total cholesterol	6.28 per cent
Free cholesterol	1.85 per cent
Phosphatid P	0.12 per cent
Total fatty acid	14.60 per cent
Neutral fat	1.26 per cent

*Cholesterol Esterase*—Sperry<sup>2</sup> has demonstrated that this enzyme in the blood is capable of esterifying the free cholesterol of the blood after incubation. In normal individuals the percentage of the free cholesterol of the blood which was esterified varied from 29 to 84 per cent. Two samples of the patient's blood plasma were analyzed\* (Sept. 23 and 25, 1935) for cholesterol esterase and the results obtained were 9 and 17 per cent esterification, respectively. Although these values are significantly lower than normal, the absolute amount of free cholesterol esterified is quite large because of the high initial concentration of cholesterol.

#### DISCUSSION

This case is of particular interest because toxic hepatitis with jaundice appeared to be responsible for the lipid imbalance associated with xanthomatosis. Bloch<sup>4</sup> has postulated that xanthomatosis is due to a disturbance in the equilibrium of the blood lipids resulting in their precipitation rather than to hypercholesteremia per se. In the present case there is not only marked lipid imbalance but also an hypercholesteremia.

Marked liver damage was suggested by the small amount of blood cholesterol esters present during the period of hospitalization. Shortly before death when the liver damage appeared to be greatest, there was a marked increase in the percentage of the cholesterol esters together with a moderate drop in the concentration of the total cholesterol. In addition, this was accompanied by the disappearance and softening of many of the lesions. Under these conditions the rôle of cholesterol is problematical.

\*We are indebted to Dr. W. M. Sperry of the Babies Hospital, New York City, for his kindness in analyzing these blood samples by his special method.<sup>20</sup>

chorio-amniotic capsule containing numerous blood vessels and easily separated from the nodules. There were several large nodules; one measured 5 by 3.5 by 2.5 cm. and looked like a small kidney. Another was double, a smaller nodule growing out from the larger one. The under one measured 5.5 cm. across, and the two protruded out from the placenta 5 cm. The largest nodule is a hemisphere 4.5 cm. in diameter, including its capsule, pearly white to pink in color, pierced by cavernous blood vessels and extending through and protruding from both sides of the placenta. This round nodule is covered by a thick capsule of chorion and over the top of the nodule in the capsule runs a large blood vessel from the cord. Besides these 8 large protruding nodules there are numer-



Fig. 4.—High power. Dark colored angiomatous area with many small and dilated capillaries

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patient The administration of betaine hydrochloride, choline hydrochloride, thyroid extract, liver extract, and insulin had little significant effect

The authors are indebted to Drs S D Blackford, H B Mulholland and D C Smith for their chemical observations in this case

## REFERENCES

- 1 Rowland, R S Anomalies of Lipid Metabolism, Oxford Medicine, Oxford University Press, New York 4 2143, 1932
- 2 Chanutin, A, and Ludewig, S The Effect of Cholesterol Ingestion on the Tissue Lipids of Rats, *J Biol Chem* 102 57, 1933
- 3 Sperry, W. M, and Stoyanoff, V A Effects of Long Continued Cholesterol Feeding in Rats, *J Nutrition* 9 131, 1933
- 4 (a) Best, C H, and Huntsman, Llnor M The Effects of the Components of Lecithine Upon the Deposition of Fat in the Liver, *J Physiol* 75 405, 1932  
(b) Best, C H, and Ridout, J H The Effects of Cholesterol and Choline on Deposition of Liver Fat, *J Physiol* 78 415, 1933
- 5 Bloch, B Metabolism, Endocrine Glands and Skin Diseases With Special Reference to Acne Vulgaris and Xanthoma, *Brit J Derm & Syph* 43 61, 1931
- 6 Schoenheimer, R Über eine Störung der Cholesterin Ausscheidung, *Ztschr f klin Med* 123 749, 1933
- 7 Thannhauser, S J Über Lipoidosen, *Klin Wchnschr* 13 161, 1934
- 8 Sperry, W M, and Schuck, B Treatment of Two Cases of Essential Xanthomatosis With Cholesterol Free Diets *Am J Dis Child* 51 1373, 1936
- 9 Hurstall, L M, and Hunt, H M Clinical Relationship of Blood Cholesterol With a Summary of Our Present Knowledge of Cholesterol Metabolism, *Ann Int Med* 9 717, 1935
- 10 Matrossowitsch, D Insulinwirkung bei einer schweren Störung des Lipidstoffwechsels, *Klin Wchnschr* 4 143, 1934
- 11 Bloor, W R The Determination of Small Amounts of Lipid in Blood Plasma, *J Biol Chem* 77 53, 1928
- 12 Okey, R A Micro Method for the Estimation of Cholesterol by Oxidation of the Digitonide, *J Biol Chem* 88 367 1930
- 13 Yasudr, M Contributions to the Micro Determination of Cholesterol, *J Biol Chem* 92 303, 1931
- 14 Man, E B, and Peters, J P Gravimetric Determination of Serum Cholesterol Adapted to the Man and Gildca Fatty Acid Method, With a Note on the Estimation of Lipoid Phosphorus, *J Biol Chem* 101 685, 1933
- 15 Osata, S, and Heki, M On the Micro Determination of Lipids in Tissues, *J Biol Chem* 87 543, 1930
- 16 Boyd E M A Differential Lipid Analysis of Blood Plasma in Normal Young Women by Micro Oxidative Methods, *J Biol Chem* 101 323, 1933
- 17 Page, I H, Kirk, E, Lewis, W H, Jr, Thompson W R, and Van Slyke, D D Plasma Lipids of Normal Men at Different Ages, *J Biol Chem* 111 613, 1935
- 18 Schoenheimer, R, and Sperry, W M A Micro Method for the Determination of Free and Combined Cholesterol, *J Biol Chem* 106 745, 1934
- 19 Epstein, E Z Cholesterol of the Blood Plasma in Hepatic and Biliary Diseases, *Arch Int Med* 50 203, 1932
- 20 Sperry, W M Cholesterol Esterase in Blood, *J Biol Chem* 111 467, 1935
- 21 Rabinowitch, I M Effects of Betaine Upon the Cholesterol and Bilirubin Contents of Blood Plasma in Diabetes Mellitus, *Canad M A J* 34 637, 1936
- 22 Cameron, G R, and Karunaratne, W A E Liver Changes in Exophthalmic Goitre, *J Path & Bact* 41 267, 1935

The highly colored urine gave a positive test for bile pigment but was otherwise essentially normal. The icterus index was recorded as 25 and 30 on two occasions. The direct and indirect van den Bergh tests were strongly positive. There was a 40 per cent retention of bromsulphthalein at the end of thirty minutes and 20 per cent at the end of an hour. The blood urea was 38 mg per cent and the fasting blood sugar was 111 mg. per cent. A glucose tolerance test was normal. The blood calcium was 9.8 mg. per cent and the blood phosphorus was 4.4 mg per cent. There were 3,400,000 red blood cells and 8,400 white blood cells. The blood smear was normal. The reticulocytes were 0.3 per cent. There was no abnormal fragility of red blood cells. The basal metabolism was normal. The cholecystogram showed a poor gallbladder shadow. Roentgenograms showed several well circumscribed areas of rarefaction in the occipital region of the skull, the largest of which measured 0.5 cm. in its greatest diameter.

The patient was on a routine hospital diet from May 14 to May 19 and was then restricted to a diet free of butter, eggs, whole milk, cream, fried foods, liver, mayonnaise, and nuts. He was allowed fruits, vegetables, salads with vegetable oil dressing, lean meat, bread, jam, coffee, tea, and skimmed milk. This diet was continued during the remainder of his period of hospital observation. Ferme ammonium citrate was given daily and sodium amytal, veronal, codeine, or luminal when necessary for insomnia. He was allowed to take mild exercise.

Five grams of betaine hydrochloride (Eastman) was given daily from June 27 to July 7. It was discontinued for one week and given again in 10 gm doses daily from July 15 to August 8. This was followed by a period free of medication until August 24, choline hydrochloride (Eastman) in 2.5 gm doses was given twice daily until September 18, when this substance had to be discontinued because of its nauseating effect. On October 23 thyroid extract was begun, 4 gr were given for three days, 2 gr for eleven days and 3 gr for ten days, the last dose being given on November 16. From November 23 to January 15, two tablespoonfuls of concentrated aqueous liver extract (Valentine) were given daily. The liver extract was supplemented then by 30 units of insulin daily for eleven days and by 45 units daily for eleven more days, to February 5. The liver extract was continued until his discharge on May 15, 1935. The patient was extremely cooperative with his diet and general medical routine.

After discharge the patient was allowed to return to unsupervised dietary regime. On Sept 2, 1935, he returned by request for observation. His clinical condition was worse. Physical examination showed the presence of fluid in the abdominal cavity, and an enlarged nodular liver. The total plasma protein was 6.38 gm per cent and the albumin:globulin ratio was 1.27.

On April 4, 1936, the patient returned again and 3,000 c.c. of ascitic fluid were obtained by abdominal paracentesis. The liver was found to be smaller, harder, and more nodular. The xanthomatous lesions were definitely smaller and many had disappeared. The total protein of the blood plasma was 6.70 gm. per cent, albumin:globulin ratio was 1.1. The fibrinogen was 0.44 gm per cent. The nonprotein nitrogen of the blood was 21 mg. per 100 c.c. and the nonprotein nitrogen of the ascitic fluid was 22 mg per 100 c.c.

The patient died at home on May 20, 1936, after an acute illness of about twenty-four hours. The details of the terminal stages could not be ascertained and permission for necropsy was not obtained.

#### CHEMICAL STUDIES OF CASE\*

**Blood Cholesterol**—The changes in concentration of total, free and combined cholesterol with respect to diet and medications are shown in Fig 5. It is seen that the esterified cholesterol is either absent or present in small quantities throughout the experiment. The highest value for total cholesterol (1480 mg.

\***Procedures** Blood lipid analyses were done on oxalated blood obtained after eighteen hours of fasting. The free and total cholesterol and the total fatty acids were determined by the oxidative method of Bloor<sup>11</sup> with modifications by Okey<sup>12</sup> and Yasuda.<sup>13</sup> The lipid phosphorus was determined according to the procedure recommended by Man and Peters.<sup>14</sup> The xanthomatous tissue was extracted by the method of Osata and Heki.<sup>15</sup> The calculations for the phosphatids esterified cholesterol, cholesterol esters, neutral fat and total lipid were done according to Boyd<sup>16</sup> and Page and associates.<sup>17</sup>

munization against the majority of the bacteria which infect the respiratory tract. Heterophile or Forssman antigen is widely distributed, apparently in rather a promiscuous fashion, among animals, plants, and bacteria. Its properties, in general, are uniform regardless of its source. Injection into rabbits excites the production of sheep cell hemolysins. Within certain limitations it possesses combining and absorptive properties not only for its homologous, but also for heterologous Forssman antibodies. The antigen is heat stable. Chemically it is a combination of lipid and protein. In some instances a carbohydrate may also be present.\*

The observations that heterophile antigen could be given orally,<sup>7</sup> that rabbits immunized orally with a pneumococcus vaccine were protected against pneumococcus pneumonia and septicemia when produced by intratracheal injection,<sup>8</sup> and that oral immunization to typhoid is successful, suggested that the oral administration of heterophile antigen would simplify immunization and make possible the frequent administration which is absolutely essential for success.

That antigens made from respiratory organisms are absorbed when given orally has been further substantiated by the work of Ross. He has shown that specific type antibodies are found in the blood of human beings who have been immunized to pneumococci orally, and also of rats, and that the rats are protected against lethal doses of virulent pneumococci.

Heterophile antigen, thus, offered a solution to the two obstacles that were not overcome in all previous studies of this nature. In it we have a single broad antigen that can be given orally, which makes frequent administration practical.

Such heterophile vaccine, made from a single strain of pneumococcus which was mostly rough "DR 1," was tried in a limited way during the winter of 1932 and 1933 by one of us (H. M. P.). We then improved the vaccine and administered it to a large group of persons during the winter of 1933 and 1934.<sup>8</sup> The vaccine was further improved and during the winter of 1934 and 1935 was given to another large group.<sup>9</sup> These studies showed a decrease in the incidence of colds among those taking the vaccine, of approximately 45 per cent.

These reports have been at least partially verified by the work of the Thomsons,<sup>10</sup> who report the prevention of colds by oral administration of respiratory vaccine. However, these investigators apparently attribute their success to type specific antibodies. They probably also obtained heterophile antibody response, and we believe this may account for at least part of their success.

Aside from these favorable reports there was at least one unfavorable criticism of the work.<sup>11</sup> In this review of our work it was stated, "the group taken as controls had, in all instances previous to the experiment, a lower average number of colds than the vaccinated group", and that there are many disadvantages attached to the use of oral vaccine, "most of which are obvious," without specifically pointing out a single one. In one of the original articles detailed information is given showing the number of colds suffered by each

\*For a review of heterophile antigen and antibody see *The Newer Knowledge of Bacteriology and Immunology*, The University of Chicago press 1928.

After four months of an unsupervised dietary regime (Sept. 23, 1935), the total and free cholesterol concentrations\* were found to be 860 and 650 mg. per cent, respectively. About six months later (April 4, 1936) the total and free blood cholesterol concentrations\* were 500 and 270 mg. per cent, respectively. The total and free cholesterol concentrations of the ascitic fluid present at this time were found to be 13.6 and 7.4 mg. per cent, respectively. It is interesting to note that the cholesterol esters were 46 per cent of the total in both blood plasma and ascitic fluid.

TABLE I  
PERCENTAGE COMPOSITION OF BLOOD PLASMA LIPIDS

FREE CHOLESTEROL %	CHOLESTEROL ESTERS %	PHOSPHATID %	NEUTRAL FAT %	TOTAL LIPID MG. %	NOTES
31	2	62	5	3139	Control
26	11	50	13	2725	Control
26	16	56	2	2388	Betaine 5 gm.
26	0	65	9	2405	Betaine 5 gm.
30	6	62	2	2281	Control
28	6	59	7	2675	Betaine 10 gm.
26	4	57	13	3077	Betaine 10 gm.
25	5	53	17	3080	Betaine 10 gm.
28	3	63	6	3092	Betaine 10 gm.
30	5	64	1	2918	Betaine 10 gm.
31	4	61	4	2642	Betaine 10 gm.
29	5	64	2	3072	Betaine 10 gm.
27	7	51	15	3107	Betaine 10 gm.
27	10	60	3	3109	Betaine 10 gm.
27	0	53	20	3174	Betaine 10 gm.
30	3	54	13	3073	Betaine 10 gm.
33	1	59	7	2755	Control
26	11	59	4	3172	Control
29	7	58	6	2667	Choline 5 gm.
25	11	56	8	2999	Choline 5 gm.
23	7	51	19	3302	Choline 5 gm.
24	6	57	13	3175	Choline 5 gm.
27	5	55	13	3154	Choline 5 gm.
29	5	62	4	2623	Choline 5 gm.
29	6	64	1	2697	Choline 5 gm.
32	5	62	1	2694	Control
31	3	63	3	2481	Control
29	6	61	4	3022	Control
30	4	62	4	3087	Thyroid
30	4	63	3	3024	Thyroid
29	8	61	2	3588	Thyroid
27	7	62	4	3760	Thyroid
30	5	59	6	3010	Control
30	5	64	1	2837	Liver extract
30	5	59	6	2721	Liver extract
29	3	64	4	3059	Liver extract
26	5	56	13	2848	Liver extract
30	2	59	9	2592	Liver extract
27	1	55	17	2662	Liver extract +
27	4	58	11	2301	Insulin
23	10	57	10	2369	Insulin
24	8	58	10	2539	Insulin
27	12	60	1	2797	Liver extract
31	1	60	8	2957	Liver extract
27	3	57	13	2956	Liver extract
8	33	33	26	592	Normal values according to Boyd <sup>14</sup>

\*These determinations were made by the method of Schoenheimer and Sperry.<sup>15</sup>

their ability to resist the effects of the gastrointestinal secretion. The bacterial cultures were sterilized with heat, the bacteria separated, absorbed on starch, dried at room temperature, and finally filled into capsules. Each capsule contained pneumococci 25 billion, *H. influenzae* 5 billion, streptococci 15 billion, and *M. catarrhalis* 5 billion.

The immunization consisted of the ingestion of one capsule with a half glass of cold water at least one half hour before breakfast, for seven consecutive mornings, after which one capsule per week was taken throughout the season.

Table I shows a summary of the data. For the sake of comparison the experiments and controls are divided into subgroups having the same average number of colds per year previous to the experimental year.

TABLE I\*

AVERAGE NUMBER OF COLDS PER PATIENT, PER YEAR, IN PAST (3 YEAR BASIS)		NUMBER OF PATIENTS	TOTAL COLDS PER YEAR IN PAST (3 YEAR BASIS)	TOTAL COLDS FOR EACH GROUP FOR EXPERIMENTAL YEAR
1	Took vaccine	4	4	3
	Control	5	5	5
2	Took vaccine	15	30	11
	Control	22	44	43
3	Took vaccine	21	57	25
	Control	22	66	67
4	Took vaccine	17	68	23
	Control	16	64	57
5	Took vaccine	12	60	13
	Control	10	50	46
6	Took vaccine	4	18	4
	Control	5	0	17
7	Took vaccine	3	21	4
	Control	2	14	12
8	Took vaccine	2	16	3
	Control	4	32	32
Continuous	Took vaccine	15		8
	Controls	14		None had continuous colds 11 patients had continuous colds 3 patients had a total of 8 colds

\*Any coryza or bronchitis whether mild or severe was counted as a cold.

In order to make an analysis of the group as a whole we have rearranged the data in Table I in a more condensed form as shown in Table II.

From Table II it will be noted that the vaccinated patients showed a decrease of 77.8 per cent in the number of colds during the experimental year as compared to the previous three years whereas the controls showed a decrease of only 10.1 per cent, making an essential decrease due to the vaccine of 67.7 per cent. If we compare the vaccinated individuals directly to the controls (this is permissible in this instance because the experimental group and the controls individually and collectively suffered approximately the same number of colds per year in the past), we find that during the experimental year the

The therapeutic measures attempted in this case were based on evidence presented in the literature. The initial response to the feeding of a "fat-free" diet, for which excellent results have been reported, was a marked drop in all the lipid constituents. After a basal level was thought to have been reached, betaine and choline were administered since Best showed that these substances could prevent the formation of fatty livers under certain conditions. There was a marked drop in the lipid constituents when betaine was first given but in view of subsequent analyses it is thought that this decrease was due to the effects of the "fat-free" diet. Continued administration of these drugs produced no marked change from an established basal level. Recently, Rabinowitch<sup>21</sup> reported that the blood cholesterol of diabetics was not significantly affected after betaine administration. However, this worker believed that carbohydrate tolerance was improved and that liver function paralleled this improvement.

Hurxthal and Hunt<sup>9</sup> have suggested that thyroid extract might have a favorable influence upon the absorption of the lesions of xanthomatosis, since it has been found that hypocholesteremia accompanies hyperthyroidism. The administration of thyroid extract in this case resulted in a marked increase in all the lipid constituents which seems to contradict Hurxthal's suggestion. The liver in this patient was probably damaged still further by the administered thyroid extract since the liver may show fatty changes and necrosis in exophthalmic goiter.<sup>22</sup>

Liver extract was given with the hope that some missing factor might be supplied to the damaged liver, but it was found to be ineffective. Insulin was injected to supplement the liver extract because of the remote possibility that it might affect the glycogen storage in the liver and thus indirectly aid lipid metabolism, but this therapy was not effective.

Xanthomatosis appears to be a condition arising from a number of different causes and has been noted in patients with and without definite metabolic disorders. Pathologic studies of the gross and microscopic picture have been exhaustive in this field, but comparatively little has been contributed to the fundamental understanding of this problem. The chemical studies in these cases have thus far yielded the most promising results for an understanding of the etiology of xanthomatosis, but they have been limited in scope. It is believed that future studies should emphasize the metabolic picture as thoroughly as possible if the problem is to be understood.

#### SUMMARY

A case of xanthomatosis with marked lipid disturbance, following arsenical hepatitis, is described.

The lipid partition of the blood plasma lipids was characterized by extreme reduction of cholesterol esters, marked reduction of neutral fat and markedly increased free cholesterol, phosphatids, and total lipids.

Treatment with a "fat-free" diet was effective in causing a moderate reduction of the lipid constituents without affecting the clinical condition of the

boys were sick in bed eighteen days less than the controls, or a decrease of 42.5 per cent. Actually the decrease was greater than indicated, for one boy among the vaccinated group came down with a bronchial pneumonia within less than two weeks after the vaccine was started. If we subtract the nine days he was ill, we find that the controls were ill in bed for forty-two days, while the vaccinated boys had only a total of fifteen days, a decrease of twenty-seven days, or 64.2 per cent.

#### SUMMARY

In the complex clinical condition usually referred to as a cold or an upper respiratory infection it is obvious that any one type of method for prevention cannot be entirely successful. Obstacles which prevented the success of prophylactic respiratory vaccines in various attempts in the past have been pointed out. The advantage of an oral vaccine high in heterophile antigen as a prophylactic in the common cold have been outlined.

Actual clinical studies on a total of 2,150 patients have been made to date. In this paper we report the results of the last 200 of these patients, the others having been reported previously. The patients in this report were studied during the winter of 1935 and 1936, while the others were studied during the winters of 1933 and 1934, and 1934 and 1935.

The 200 patients reported in this paper consisted of 100 who took the vaccine and 100 who acted as controls. The controls had approximately four times as many colds during the experimental year as those who took the oral vaccine. There was also a very marked decrease in the days of illness from all causes among the vaccinated group as compared with the controls.

#### REFERENCES

- 1 Kruse, W. Die Erreger von Husten und Schnupfen, *München med Wchnschr* 61 1547, 1914.
- Shibley, G. S., Mills, K. C., and Dochez, A. R. A Study of Acute Infection of the Respiratory Tract in the Ape, *Proc Soc Exper Biol & Med* 26 562, 1929.
- Further Consideration of Transmissibility of Human Upper Respiratory Infections (common cold) to the Ape, *Ibid* 27 59, 1930.
- Dochez, A. R., Mills, K. C., and Kneeland, Y., Jr. Study of the Virus of Common Cold and Its Cultivation in Tissue Medium, *Ibid* 28 513, 1931.
- Cultivation of the Virus of Common Cold in Tissue Medium, *Ibid* 29 64, 1932.
- Powell, H. M., and Clowes, G. H. A. Cultivation of the Virus of Common Cold and Its Inoculation in Human Subjects, *Ibid* 29 332, 1931.
- 2 Smith, W. Discussion on the Routes of Infection and Paths of Transmission of Viruses, *Proc Roy Soc Med* 29 576, 1936.
- 3 Bailey, G. H., and Shorh, M. S. Heterophile Antigen in Pneumococci, *Am J Hyg* 13 831, 1931.
- Shorh, M. S., and Bailey, G. H. Heterophile Antigen in Various Bacterial Species, *Ibid* 19 148, 1934.
- Rockwell, G. E., and Van Kirk, H. C. The Production of Heterophile Antigen by Certain Bacteria and Plants, *J Infect Dis* 59 171, 1936.
- 4 Powell, H. M., Jameson, W. A., Buley, G. H., and Hyde, R. R. A Comparative Study of Antipneumococcus Serum Containing Heterophile Antibody, *Am J Hyg* 17 102, 1933.
- 5 Powell, H. M. Immunization With Heterophile Antigen When Given by Mouth, *Ibid* 5 228, 1925.
- 6 Kolmer, J. A., and Rule, A. M. Oral Immunization of Rabbits Against Pneumococcus Pneumonia and Septicemia, *Proc Soc Exper Biol & Med* 30 107, 1932.
- Kolmer, J. A., and Amano, K. W. Specific Prophylaxis of Pneumococcus and Streptococcus Meningitis. Vaccine Prophylaxis, *Arch Otolaryng* 15 547, 1932.

## FURTHER STUDIES ON ORAL IMMUNIZATION TO COLDS\*

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IN RECENT years the various studies on "cold virus"<sup>1</sup> have popularized the belief that colds are caused altogether by an ultra-microscopic virus. Hence, it is but natural that attempts should be made to prevent colds by immunization to this virus. But all attempts at either human or animal immunization have met with very little success. This failure may be accounted for by three factors: the weak antigenic power of the cold virus; the probability that it is not responsible for all colds; and, finally, the possibility, by analogy with influenza virus as suggested by Wilson Smith,<sup>2</sup> that, instead of the virus paving the way for the secondary invader, it actually rides in on the wave of bacterial infection.

The complex condition referred to as a "cold" or "upper respiratory infection" is far from a clinical entity. Realization of this is forced upon us when we consider the innumerable factors which play a rôle in bringing this condition about. Among them may be mentioned infections, allergic reactions, virus, diet, vitamins, fatigue, oxygen uptake and capacity, temperature changes, humidity, and distant foci of infection which drain the vitality of the body.

Whether or not the bacterial infection is primary, it still remains the factor which causes the severe illnesses and complications. Hence, any method which will prevent the bacterial respiratory infections is bound to be rewarded with a reasonable amount of success. Attempts have been made in the past to immunize against bacteria infecting the respiratory tract, but all such attempts encountered two obstacles which they were not able to overcome. The first was the fact that such a large number of bacterial species, such as the pneumococci (32 or more distinct types), *H. influenzae*, streptococci, *M. catarrhalis*, staphylococci, and many others, infect the respiratory tract, that type specific immunization necessitated the use of a mixture of so many different types of antigens that none of them could be effective.

The second obstacle was that such types of low-grade immunity are very short-lived, and hence necessitate almost continuous immunization during the year. The hypodermic method of administration made this a tedious and expensive procedure.

The discovery that pneumococci, some strains of streptococci, *M. catarrhalis*, *H. influenzae* (smooth strains), and *B. mucosus capsulatus* contain heterophile antigen,<sup>3</sup> together with the observation that heterophile antibodies confer demonstrable protection against pneumococcic infections,<sup>4</sup> paved the way for the use of a single broad antigen (heterophile) which gave promise to the widest im-

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Since the results of this study can only be presented in a very brief form, it was thought best to give Table I which shows the cumulative percentages for the entire tablets passed from the stomach in the given time. The table presents these data for the four diets and the three meals.

TABLE I

HOURS	CARBOHYDRATE			CELLULOSE		
	BREAKFAST	LUNCHEON	DINNER	BREAKFAST	LUNCHEON	DINNER
10	2 02					
15	6 06					
20	22 72	10 78	10 10	12 50		
25	27 77	18 62	25 25	66 66	1 66	1 92
30	51 00	30 38	33 33	66 66	1 66	1 92
35	54 03	41 16	33 33	72 21	11 66	15 39
40	63 12	48 02	40 40	74 98	61 66	15 39
45	66 15	48 02	41 41	81 92	61 66	21 16
50	81 30	48 02	42 42	83 31	61 66	21 16
55	81 30	48 02	42 42	84 70	61 66	28 85
60	87 36	60 76	43 43	84 70	61 66	32 69
65	88 37	60 76	43 43	93 03	66 66	72 69
70	93 92	60 76	46 46	93 03		
75	93 92	63 70	46 46	95 80		
80	93 92	75 46				
85	94 93	75 46				
90	94 93	86 24				

HOURS	FAT			PROTEIN		
	BREAKFAST	LUNCHEON	DINNER	BREAKFAST	LUNCHEON	DINNER
15					10 00	
20	4 54	11 42		17 02	20 00	14 28
25	6 81	22 84		21 27	55 00	17 85
30	9 08	28 55		21 27	55 00	24 99
35	9 08	28 55		36 16	55 00	24 99
40	9 08	39 97		51 05	55 00	24 99
45	20 44	39 97		57 4	55 00	24 99
50	27 25	39 97		61 68	60 00	24 99
55	29 52	39 97		65 93		24 99
60	31 79	48 54		65 93		24 99
65	36 33	48 54		80 82		28 56
70	36 33	62 82		80 82		35 70
75	36 33	62 82		80 82		
80	36 33	65 66	9 30	85 07		
85	36 33	65 66	9 30			
90	36 33	71 37	18 60			
95	38 60	71 37				
100	38 60	79 94				
105	38 60	79 94				
110	38 60	79 94				
115	38 60	79 94				
120	45 41	91 36				

It will be noted from the table that considering the diets as a whole, there appeared to be an increase in the length of time tablets would remain in the stomach as the day progresses. This was no doubt due to fatigue and a slowing of peristalsis. Diets high in carbohydrate or cellulose were more conducive to rapid passing of the tablets from the stomach, as was shown by the higher percentage of tablets expelled. Fat diets were the least efficient and the only type that did not follow the order set by the other three, since the highest percentage passed in the case of the luncheon. The cellulose diets which contained a high content of bran caused the greatest disintegration of the tablets in the stomach.

of the experimental groups, and by each of the controls, in the previous three years and during the experimental years. For the sake of a fair comparison, these were divided into subgroups of experimentals and controls having the same average number of colds per year previous to the experimental year. However, the total average number of colds per year in the past was less when the entire control group was combined as compared to the experimental group. This could not be avoided, because in taking such a group as the employees of one company, volunteers had to be solicited, and naturally those who were the severest sufferers from colds were the ones to volunteer. In order to have our controls working under the same conditions it was necessary to take the other employees as controls. But during the experimental year, other factors being equal, one would expect, if the vaccine were ineffective, that the controls would have fewer colds than the experimental group. Instead, the experimental group had many less colds than the control group, 40.6 per cent less for the winter of 1933 and 1934 and 35.2 per cent less for the winter of 1934 and 1935. These figures compare favorably with those arrived at by the method of calculation used in previous reports.

It is questionable if there are any serious objections to the oral administration of a respiratory vaccine. Any objections that might be valid are far overshadowed by the many advantages of this method, some of which are: ease of administration, making practical frequent administration, absence of sensitization of the individual, nonproduction of a negative phase,<sup>12</sup> a lower cost of vaccination, and the simplicity and low cost combining to encourage mass immunization with all of its benefits.

The experiments on animal protection,<sup>13</sup> and the studies on 191 patients which show a very definite relation between the patients' development of heterophile immunity and their incidence of colds<sup>14</sup> is further proof of the effectiveness and value of oral immunization with a respiratory vaccine high in heterophile antigen.

During the winter of 1935 and 1936, we again gave the vaccine to a group of patients and observed another group for controls. These groups consisted of 100 persons who took the vaccine and 100 persons who were used as controls; making a total of 200 persons who were observed in this study. Because these groups were much smaller than the ones studied in previous years, we were able to avoid some of the difficulties which we had previously encountered.

#### METHOD

The patients who participated in this study came from various walks of life in Cincinnati and Greenville, Ohio. They consisted of boys in an institution, medical students, office workers, factory workers, and school children. In each group half the persons took the vaccine, while an identical number acted as controls. We were thus able to select the controls so that they were as nearly identical as possible with those taking the vaccine, with respect to age, occupation, environment, and number of colds suffered per year in the past, on the basis of a three-year average.

The oral vaccine consisted of a mixture of bacteria which infect the respiratory tract. The strains were selected for their heterophile content, and

## THE PRODUCTION OF CHRONIC ARTHRITIS BY INDOLE AND OTHER PRODUCTS OF TRYPTOPHANE PUTREFACTION\*

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IT HAS been reported by the authors<sup>1</sup> that indole, a product of tryptophane decomposition, is usually present in the urine of patients having rheumatoid arthritis. In a more recent paper,<sup>2</sup> it was shown that this indoluria diminishes with clinical improvement and finally disappears with recovery. These findings suggested that indole might be a factor in the causation of an arthritic joint. It was decided to ascertain whether or not indole, or other products of tryptophane decomposition, would produce, when injected into the joint cavity, any pathologic changes similar to those found in arthritis. The present paper deals with the results obtained in this investigation.

It has been shown by Axhausen,<sup>3</sup> Key,<sup>4</sup> and Burekhardt<sup>5</sup> that arthritis like changes can be produced by the injection of a small amount of a strong chemical irritant, such as tincture of iodine or carbolic acid into the joint cavity. Seeliger<sup>6</sup> produced similar changes in the knee joint of rabbits by the repeated injections of N/50 hydrochloric acid, and believed that the acid reaction within the joint was responsible for the arthritis. Habler<sup>7</sup> repeated Seeliger's experiments and obtained comparable results, but he also produced the same arthritic changes in rabbits by numerous injections of distilled water into the knee joints. Key<sup>8</sup> confirmed Habler's results with distilled water and further showed that the injection of physiologic saline would cause similar lesions. The changes produced by these milder irritants were not severe, being very slight compared with those which are here reported from the injection of indole and related compounds.

*Experimental*—Adult rabbits, without regard to size, sex or breed, were used as experimental animals. Sterile solutions of indole, skatole, indole, propionic acid and tryptamine (B indolethylamine) were injected aseptically into the joint cavity of one hind knee. Except where otherwise stated, a 40 per cent aqueous solution of diethylene glycol was used as a solvent for these compounds, and the injections were repeated once or twice a week for nine weeks.

*Indole Experiment 1* Eight rabbits received intracapsular injection of 10 mg. of indole in 1 c.c. of the solvent solution, 2 received 5 mg. in 0.5 c.c., and 2 received 3 mg. in 0.3 c.c. Equal amounts of the solvent solution were injected into the opposite knee joints. The injections were repeated once a week for nine weeks. With one exception all of the rabbits were killed within two weeks after the last injection. Sacrifice of the last rabbit was delayed for five weeks to allow further changes to take place in the joint. Fig. 1 shows

\*From the Department of Biochemistry, Medical College of Virginia.  
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controls had 375 colds whereas the vaccinated patients had a total of only 94 colds, a decrease of 281 colds or 74.9 per cent. In other words the controls had four times as many colds as the vaccinated patients.

TABLE II

	NUMBER OF PATIENTS	TOTAL COLDS* PER YEAR IN PAST (AV. 3 YR. BASIS)	TOTAL COLDS* IN EXPERI- MENTAL YEAR	DECREASE IN NUMBER OF COLDS	PER CENT DECREASE
Controls	100	417	375	42	10.1
Took oral vaccine	100	424	94	330	77.8
Essential decrease due to vaccine					67.7

\*Patients reporting continuous colds were calculated as having 8 colds per year.

Among the above reported groups were boys from an institution. Because all of the boys in this institution were included in this work, half receiving the vaccine and the other half acting as controls, it is of interest to analyze their results separately. The data for these boys are shown in Table III. These boys lived, ate, went to school, and played together.

TABLE III

## BOYS' INSTITUTION

BOYS TAKING ORAL COLD VACCINE				BOYS NOT TAKING VACCINE BUT USED AS CONTROLS			
NAME	TOTAL NO. COLDS WINTER OF 1934 AND 1935	TOTAL NO. COLDS WINTER OF 1935 AND 1936 EXPERIMENTAL WINTER	TOTAL DAYS' ILLNESS* DUE TO ALL CAUSES	NAME	TOTAL NO. COLDS WINTER OF 1934 AND 1935	TOTAL NO. COLDS WINTER OF 1935 AND 1936 EXPERIMENTAL WINTER	TOTAL DAYS' ILLNESS* DUE TO ALL CAUSES
C. B.	5	1	9	R. C.	4	4	6
M. B.	Continuous	0	0	R. G.	3	4	5
M. O.	4	2	0	G. G.	1	1	0
E. H.	Continuous	0	0	C. H.	1	1	3
B. S.	1	1	0	L. L.	1	2	6
N. B.	Continuous	0	10	W. L.	2	4	3
J. B.	4	1	5	E. L.	3	2	4
G. S.	Continuous	0	0	D. S.	1	0	0
J. C.	2	1	0	T. S.	2	3	10
S. B.	3	1	0	G. S.	2	3	5
Total	-	7	24	Total	-	24	42

\*Unable to attend to daily duties.

From Table III it will be seen that the ten boys receiving the vaccine had a total of only 7 colds during the experimental year while the ten boys acting as controls had a total of 24 colds. The vaccinated boys had 17 less colds than the controls, or a decrease of 70.8 per cent. It will also be noted that the vaccinated boys were sick in bed, from all causes, a total of only twenty-four days, while the controls were confined to bed forty-two days. Thus the vaccinated

wasting of the thigh muscles above the indole treated joint, and marked crepitus was evident on manipulation. In opening the joint it was necessary to cut through approximately 4 mm of dense fibrous tissue. The cavity contained apparently normal amounts of synovial fluid, and showed no evidence of infection, only a few pus cells being evident on microscopic examination. The control joints showed no gross deviation from normal.

Microscopic examination of the indole treated joint revealed marked proliferative activity of the articular cartilage, with cellular arrangement into vertical rows. In places the cartilage was thinned and deeply eroded. The thinned and eroded surfaces were covered with granulation and fibrous tissues which were replacing the cartilage and bone. The patellar surfaces showed the same changes. The epiphyseal cartilage showed no apparent change. There were marked increased fibrosis and thickening of the capsular attachments. The microscopic appearance of the control joint exhibited no abnormal changes.

*Skatole Experiment* With the same procedure as used in the indole experiments, two rabbits were given injections of 5.6 mg of skatole (the molecular equivalent of 5.0 mg of indole). Two more received injections of 2.8 mg of skatole plus 2.5 mg of indole. The substances were dissolved in 40 per cent diethylene glycol solution and 1 cc injected. Injections were made twice weekly for nine weeks and the animals were killed one week following the last injection. Grossly, the joints receiving the skatole and the skatole and indole, were similar in appearance to those receiving comparable amounts of indole for the same length of time, though possibly the changes were not quite so severe.

*Indole Propionic Acid Experiment* Two rabbits were used in the experiment on this indole derivative, 8.1 mg of indole propionic acid (the molecular equivalent of 5.0 mg of indole) were injected twice a week for nine weeks. The rabbits were killed five days following the day of the last injection. The changes in the joints receiving the indole propionic acid were similar to those produced by indole. The cartilage destruction was about the same, but the proliferation of fibrous tissue was somewhat less in the indole propionic acid treated animals.

*Tryptamine Experiment* Two rabbits were given injections of 10 mg of tryptamine dissolved in 1 cc of saline. One cubic centimeter of saline was also injected into the control joints. Injections were made weekly for twelve weeks and the animals were killed one week later. At autopsy the joints showed no gross evidence of any arthritic changes in either the tryptamine or the control joint. The difference between our results with saline and those of Key<sup>8</sup> is undoubtedly due to the fact that we gave so few injections.

The experiment was repeated, using 10 mg of tryptamine, but 40 per cent diethylene glycol solvent solution was used instead of normal saline. The solvent solution was also injected into the control joints. Injections were made biweekly. The rabbits were killed by mistake at the end of five weeks. Autopsy of the knee joints showed no apparent differences between the tryptamine injected and the control joints.

7. Ross, V.: Duration of the Immunity Produced in Rats by Feeding the Pneumococcus Type I, *J. Immunol.* 27: 235, 1934.  
Protective Antibodies Following Oral Administration of Pneumococcus Type I to Rats, *Ibid.* 27: 249, 1934.  
Protective Antibodies Following Oral Immunization of Pneumococcus Types II and III to Rats, With Some Data for Types IV, V, and VI, *Ibid.* 27: 273, 1934.  
Oral Immunization of Humans Against the Pneumococcus, *Ibid.* 27: 307, 1934.
8. Rockwell, G. E., Van Kirk, H. C., and Powell, H. M.: Oral Immunization to Colds, *Ibid.* 28: 475, 1935.
9. Rockwell, G. E., Van Kirk, H. C., and Powell, H. M.: Further Studies on Oral Immunization to Colds, *Science* 82: 177, 1935.
10. Thomson, D., Thomson, R., and Thompson, E. T.: Immunization by the Oral Route in Respiratory Infections, *Brit. M. J.* 1: 258, 1936.
11. Editorial, Oral Immunization to Colds, *J. A. M. A.* 105: 804, 1935.
12. Powell, H. M.: On the Non Appearance of the Negative Phase in Treatment With Heterophile Antigen by Mouth, *Proc. Ind. Acad. Sc.* 34: 261, 1925.
13. Rockwell, G. E., and Van Kirk, H. C.: Oral Heterophile Immunization, *J. Bact.* 29: 47, 1935.
14. Rockwell, G. E., and Van Kirk, H. C.: The Relation of Heterophile Immunity to the Incidence of Colds, *J. Immunol.* 28: 485, 1935.  
Rockwell, G. E., and Van Kirk, H. C.: Further Studies on the Relation of Heterophile Immunity to the Incidence of Colds, *Ibid.* 31: 417, 1936.

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## THE EFFECT OF DIET ON ENTERIC COATED TABLETS\*

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**I**N CONNECTION with previous studies on enteric coatings, by the authors and other investigators, the question of the influence of diet on the time which enteric coated tablets remain in the stomach naturally arose. It was, therefore, decided to make a study of this problem using diets consisting of carbohydrate, cellulose, fat, or protein. The percentage of the principal constituent was varied over a wide range, in order to observe what effect, if any, this factor might have on the time elapsing before the tablets were expelled. The test meals were also varied in amount with the individual subject, in order to ascertain whether the size of the meal had any influence. The effect of the time of day at which the meal was eaten and the amount of water consumed were also observed.

The study was conducted using the x-ray in order to remove all element of doubt concerning the location of the tablets in the body. For this purpose, tablets of barium sulphate were coated with a synthetic resin† which had been previously tested and found to be the most efficient type of enteric coating investigated by the authors. Four of these tablets were given to a subject, who was instructed to take them just before the test meal. Radiographs were taken at about two-hour intervals until most of the tablets had passed from the stomach. The subjects used in this study were students of the College of Pharmacy, University of Nebraska. These individuals took a total of 802 tablets in the 204 experiments that were conducted. Six hundred fifty-eight radiographs were taken.

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\*From the College of Pharmacy, University of Nebraska.

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†Tablets used in these experiments were supplied by the Abbott Laboratories.

# THE EFFECT OF ANTIPERNICIOUS ANEMIA SUBSTANCES UPON GUINEA PIG RETICULOCYTOSIS AND A REVIEW OF THE LITERATURE\*

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A CHARACTERISTIC and specific reticulocytosis in the guinea pig from substances effective in the treatment of pernicious anemia has been recently reported by Jacobson<sup>11 12</sup> This report pertains to our findings in a similar study.

Male guinea pigs weighing approximately 500 gm were used They were kept in wire bottom cages The animals were fed oats ad lib, carrots and lettuce, at the same time each day No water was given

The reticulocyte counts were made daily except on Sunday, by the same person and at the same time each day The reticulocyte counts were estimated according to the method of Landsberg and Thompson<sup>14</sup>

In each determination a minimum of 500 cells was counted This method was found to be simple, satisfactory, and accurate Repeated determinations convinced us that the results compared closely with those obtained when the Jacobson method was used This technique had the added advantage of making the reticulum more distinct, thus reducing the error

After a suitable control period the material under investigation was administered by the designated route Reticulocyte counts were then repeated for an adequate period of time Orally the material was given by medicine dropper, and intraperitoneal injections were accomplished by inserting a hypodermic needle directly into the abdomen at an angle of 45 degrees

Limited space makes it impossible to present here all of the counts made from day to day in the various experiments The complete results for a small number of guinea pigs representative of each group are, therefore, tabulated

Reticulocyte studies were first made on a group of 14 guinea pigs Control counts were made on all animals during the first week and with two exceptions the percentage of reticulocytes was well below 1 per cent There was a fair degree of uniformity throughout the control period At the end of the first week the animals were injected intraperitoneally with 5 cc of a solution of Lilly's Liver Extract No 343, each dose containing the equivalent of 5 mg of fresh liver One guinea pig died the next day In the remaining 13 animals, 1 showed a response greater than 14 per cent, reaching 30 and 38 per cent on the second and third days, respectively, of the observation period (Fig 1, Fig 7) With this single exception the only difference between the reticulocyte counts

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These results indicate that the best type of diet to insure rapid passing of the tablets from the stomach would be a mixture of carbohydrate and cellulose.

The quantity of food consumed had no apparent effect on the time the tablets remained in the stomach. This was demonstrated by the results on two subjects having the same type of diet. One of these individuals had a meal of 19.5 gm., the other 658 gm. The tablets were all passed in seven hours by the first individual and in six hours by the second. This same general trend was noted through all the experiments. The results were somewhat different where large quantities of water were ingested. An excess of fluid seemed to retard the passing of the tablets. One subject drank 4,140 c.c. of water during the experiment and retained all of the tablets in the stomach for sixteen hours. It was noted that the subjects ingesting 700 c.c. or less of liquid passed the tablets more rapidly.

Another interesting point brought out in this study was the individual's variation. It was found that the same individual did not react the same on different days with respect to the passing of the tablets although given identical diets. The same was found to be true of different individuals having the same diet.

The average time in hours based on the total number of tablets passed from the stomach, was as follows:

	BREAKFAST	LUNCHEON	DINNER
Carbohydrate	3.6+	5.0	3.2-
Cellulose	2.7+	3.3+	3.8+
Fat	5.8+	6.1+	8.5
Protein	4.2+	3.4-	3.7

The average time in hours determined in this manner does not give a true picture of the end-results as it will be noted that a lower percentage of tablets passed out of the stomach in the case of the luncheon and dinner. If the experiments could have been extended until all of the tablets had been expelled, the average time would have been increased for these two meals. Since we were using students for subjects, it was impossible to keep them all night in order to complete the experiments. However, the above values are of interest as most previous investigators have reported two to four hours as the average length of time tablets will remain in the stomach.

during the week following injection of Lilly's liver extract and those of the control period was a slightly greater tendency toward fluctuation in percentage of reticulocytes from day to day

In order to determine whether a larger dose of liver extract would give a better response, the guinea pigs were injected at the beginning of the third week with 5 cc of a solution of the same extract, each dose now containing the equivalent of 50 mg of fresh liver. During this third week of observation none of the guinea pigs, including the one which had previously responded to the 5 mg dose, showed a definitely positive response. 8 of the animals continued at the same reticulocyte level as during the control period. The remaining 5 reached 12 per cent or above on one or more days during the week following injection. Only one of this group went above 14 per cent at any time, reaching 28 per cent on the fourth day. These 5 animals were retained as being "possibly reactive" and were reserved for further study. The remaining 8 were discarded.

For a period of one week, control counts were made on the 5 guinea pigs selected as described above. At the end of this time the animals were fed an extract of pressed stomach linings prepared according to the method of Klein and Wilkinson<sup>13</sup>. During the following week there was no response in any of these animals to the administration of the stomach material. These animals were then followed for four weeks to determine their reticulocyte values without further injection. The results on three of these animals are shown graphically in Fig. 1.

If we assume that a positive response consists of a rise to 2 per cent on two consecutive days, or on two days separated by one day, and if a "weakly positive" response is assumed to be a rise to at least 2 per cent on one day, it is noted from Fig. 1 that occasional spontaneous "responses" occurred during the control period. Moreover when the four surviving pigs were injected intraperitoneally with the equivalent of 2 mg of fresh liver and studied for a final week, there was no increase in reticulocyte count which could be considered significant.

Table I illustrates typical findings in 3 animals from a group of 8 guinea pigs which were studied for seven weeks. From this experiment the following observations could be made:

1. None of the animals responded to a dose of 50 mg of fresh liver, although Guinea Pig 21 showed counts above 20 per cent, but had been at a high level during the control period.

2. Three guinea pigs exhibited a weakly positive response to 500 mg of liver, but subsequently failed to respond positively to a 2000 mg dose three weeks later.

3. One guinea pig (No. 26) showed an apparently spontaneous reticulocytosis during control periods.

4. None of the animals responded positively to a dose of 2000 mg equivalent of fresh liver.

Table II summarizes the results in 4 of another group of 18 guinea pigs studied for nine weeks. At the beginning of the observation period, Pig 36 had

the joint surfaces of this animal. The difference between the experimental and control joints of the other rabbits used in this experiment was entirely comparable with those shown in Fig. 1, taking into consideration the amount of indole injected and the duration of the experiment.

*Indole Experiment 2:* Two animals were used. Each received injections of 5 mg. of indole *twice* a week for nine weeks, and was killed five weeks after the last injection. The results were in complete agreement with those obtained in the previous experiment, the severity of the lesion being apparently the same as produced by single injections of 10 mg. weekly over the same period of time.

All joints receiving single injections of 10 mg. of indole weekly, or 5 mg. biweekly, showed definite enlargement in about ten days after the first injection.

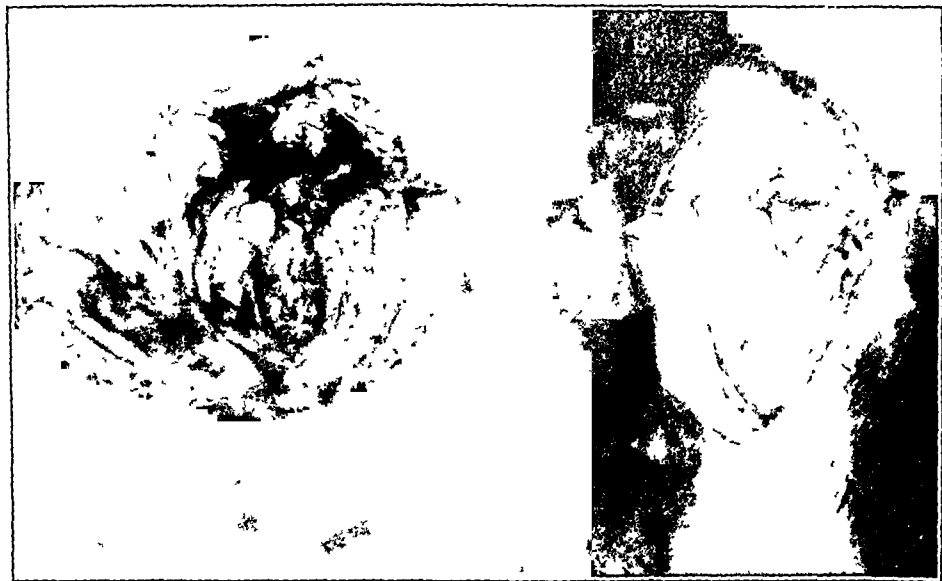


Fig. 1.—Left joint was injected with 10 mg. of indole in 40 per cent diethylene glycol once a week for nine weeks and the rabbit was killed five weeks thereafter. Right joint is the control joint. It was injected with the solvent alone for the same period of time.

tion. Crepitus could be elicited after about two weeks. After six weeks, a decided decrease in joint motility was evident. None of these symptoms was apparent in the control joints. The extent of the pathologic change was naturally greater in those animals, the killing of which was delayed for an extended period after the last injection. A detailed description of these latter joints only is here recorded, since the purpose of this paper is not a study of the changes involved in chronic arthritis, but to show that indole and other products of tryptophane putrefaction can bring about very severe arthritic lesions.

*Gross Description:* The difference between the indole-treated and control joints was always very marked, the experimental joint being about 50 per cent larger than the corresponding control joint. There was considerable

As suggested by Jacobson,<sup>11</sup> it is possible that these guinea pigs might be in a refractory period and might subsequently become reactive. They were rested for approximately six weeks before being subjected to a further study. The control period consisted of five consecutive weeks during which time three of the animals showed a definitely spontaneous response and six showed a weakly positive response, despite the fact that during this period no injections were given. We were, however, satisfied that the control counts were sufficiently consistent to permit ready recognition of any definite response to the administration of the antipermeious anemia material. At the end of this five week control period, each pig was injected intraperitoneally with  $1\frac{1}{2}$  cc of Lilly's liver extract solution, the dose being the equivalent to 2.5 gm of fresh liver. The subsequent daily reticulocyte counts revealed a positive response in only one guinea pig. However, this animal became very sick coincident with the reticulocyte rise and died on the day following the peak of the reticulocyte curve.

An L tyrosin fraction which was isolated from liver was found<sup>10</sup> by guinea pig assay to contain over 10 000 000 guinea pig units per gram. The results following the injections of 2 mg. of L tyrosin can be seen in Table III. These animals were studied for fourteen days, and three guinea pigs exhibited positive responses, returning again to normal at the end of the two week period. It may be noteworthy that none of these three guinea pigs had ever exhibited a reticulocytosis of positive significance either during the control period or following injection of liver. These results suggested repetition of the experiment, but in the same animals the same dose of L tyrosin intraperitoneally now failed to elicit a single positive response. Finally the animals were given daily intraperitoneal injections of 5 cc of Lilly's liver extract each dose containing the equivalent of 1 gm of fresh liver. The detailed findings were entirely negative.

#### COMMENT

Jacobson's findings<sup>11, 12</sup> suggest that the guinea pig properly controlled, may serve as an assay animal for determining the antipermeious anemia potency of liver extracts. The independent report of Landsberg and Thompson<sup>14</sup> further substantiates such a conclusion. Moreover, these latter authors obtained positive findings without the meticulous adherence to conditions as specified by Jacobson concerning housing, diet of the animals and technique of reticulocyte counting. In addition, Miller and Rhoads,<sup>15</sup> despite disregard of Jacobson's stipulations, were able to obtain increased reticulocyte counts in their guinea pigs with daily feedings of 0.6 gm of Lilly's liver extract. Their responses occurred between the seventh and twelfth days, a finding which is in disagreement with Jacobson,<sup>11</sup> "that the reticulocytosis that the oral administration of liver extract induces differs qualitatively in no way from that following the intraperitoneal administration." Since Miller and Rhoads fed 500 mg daily (about 75 times the minimal effective oral dose recorded by Jacobson) their reticulocyte responses should have been obtained within six days after the first dose if their work is to be considered in full agreement with that of Jacobson.

Clark and Coene,<sup>5</sup> in the abstract of a paper read before the American Society of Biological Chemists summarized their work on this problem as follows:

## DISCUSSION

The use of indole and some of its derivatives for the production of experimental arthritis was a direct outgrowth of the finding of indole either free or in a loosely bound form in the urine of most cases of rheumatoid arthritis. Although the results of the present experiments do not prove that products of tryptophane putrefaction are causative agents in the production of rheumatoid arthritis in man, they are suggestive and would seem to justify further investigation. The mechanism through which these products bring about arthritic changes in the joints of rabbits is by no means clear. Their action is not due to the indole ring per se, since tryptamine (B-indolethylamine) produces no demonstrable lesions.

## SUMMARY

*Chronic* arthritis with extensive joint changes has been produced by the intracapsular injection of indole, skatole, and indole-propionic acid into the knee joints of rabbits. Control joints injected with equal amounts of the solvent solutions used with the above compounds failed to produce any apparent changes. Tryptamine (B-indolethylamine) had no detectable effect on the joint tissues.

The authors acknowledge with gratitude the many helpful criticisms and suggestions of Dr. Frank Apperly, Professor of Pathology, in carrying on this work and in the preparation of the manuscript. They are also indebted to Eli Lilly and Company for a research scholarship to one of us, R. C. Neale, and to the Valentine Research Fund of the College for a research grant to help defray the cost of the investigation.

## REFERENCES

1. Forbes, J. C., and Neale, R. C.: Studies on Indoluria, *J. LAB. & CLIN. MED.* 20: 1017, 1935.
2. Forbes, J. C., Neale, R. C., Hite, O. L., Armistead, D. B., and Rucker, S. L.: Studies on the Effect of a High-Sulfur Low-Carbohydrate Diet in Chronic Arthritis, *J. LAB. & CLIN. MED.* 21: 1036, 1936.
3. Axhausen, G.: Kritisches und Experimentelles zur Genese der Arthritis Deformans, insbesondere über die Bedeutung der Aseptischen Knochen-und Knorpelnekrose, *Arch. f. Klin. Chir.* 94: 331, 1911.
4. Key, J. A.: Pathological and Experimental Observations on Hypertrophic Arthritis, *Am. Med.* 23: 610, 1930.
5. Burckhardt, H.: Experimentelle Untersuchungen über die Beziehungen der Gelenkfunktion zur Arthritis Deformans, *Arch. f. Klin. Chir.* 132: 706, 1924.
6. Seeliger, P.: Ein Beitrag zur Pathologischen Physiologie der Gelenke im Hinblick auf die Arthritis Deformans, *Deutsche Ztschr. f. Chir.* 198: 11, 1926.
7. Habler, C.: Zur Frage der Aktuellen Reaktion der Gelenkexsudate und der Technik ihrer Messung, und zur Frage der Saurewirkung als Ursache der Arthritis Deformans, *Deutsche Ztschr. f. Chir.* 209: 211, 1929.
8. Key, J. A.: The Production of Chronic Arthritis by the Injection of Weak Acids, Alkalies, Distilled Water, and Salt Solutions Into Joints, *J. Bone & Joint Surg.* 31: 67, 1933.

TABLE II—CONT'D

GUINEA PIG	30	36	37	42
7	00	02	02	04
8	00	06	04	24
9				
10	04	04	00	10
11	02	09	08	00
12	04	14	04	02
13	08	06	02	00
14	10	06	06	00

"The content of the antianemic substance present in different liver extracts has been estimated by the guinea pig method of bio assay. Although the method may not be specific, reproducible results are obtained.

As far as we have been able to determine no substitute for the clinical method of liver assay has as yet stood the test of time. In 1930, Vaughan, Muller, and Minot<sup>21</sup> reported that normal grain fed pigeons responded specifically to potent liver preparations. This work was extended and supported by

TABLE III

GUINEA PIG	31	32	42	44
<i>Day</i>	<i>2 mg L Tyrosin Intraperitoneally</i>			
1	02	04	04	14
2	00	08	20	14
3	02	06	34	24
4	06	04	48	08
5	12	14	34	20
6	04	12	36	24
7	06	08	18	44
8	18	22	06	28
9	12	20	02	42
10	22	20	02	50
11	06	18	04	36
12	12	12	06	26
14	02	06	04	12
	<i>2 mg L Tyrosin Intraperitoneally</i>			
1	02	06	04	02
2	02	06	00	06
3	06	02	02	02
4	02	04	00	00
5	02	02	02	00
6	02	00	10	02
7	02	04	14	04
8	00	06	08	14
9	04	04	06	04
10	04	02	08	02
11	00	06	04	04
12	00	02	02	02
13	00	02	02	04
14	04	02	00	02
	<i>Daily injections of 1 gm equivalent of fresh liver made from Lilly's Powder</i>			
1	04	00	02	02
2	02	02	00	02
3	00	02	00	04
4	02	02	02	04
5	02	00	00	02
6	02	00	02	02
7	02	00	06	02

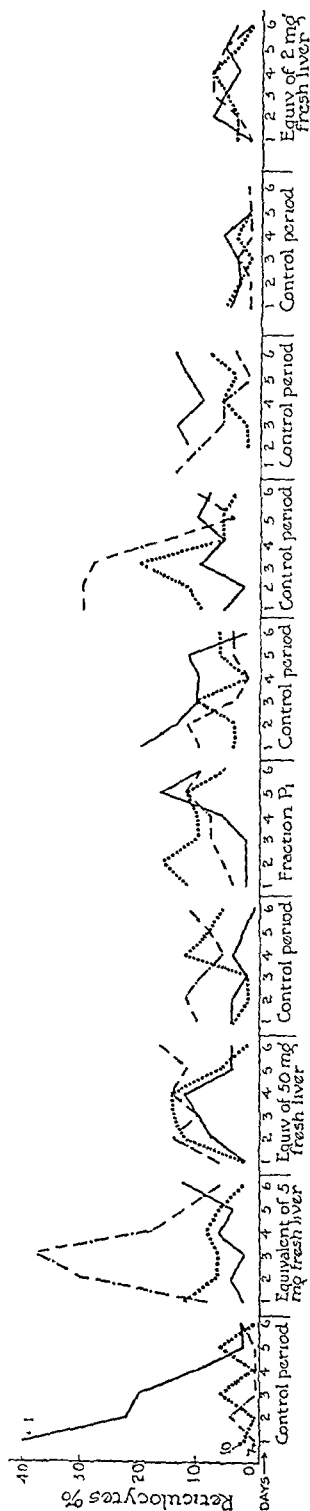


Fig. 1.—Reticulocyte counts of three typical guinea pigs during control and test periods.

respond to antianemic substances because its bone marrow is predominantly erythroblastic and normoblastic rather than megakaryoblastic which characterizes the human pernicious anemia patient. Their hypothesis was borne out by their experimental findings, neither raw liver nor liver extracts were able to evoke a reticulocyte response in rats. Gebhardt and Carro<sup>7</sup> three months later published the results of an elaborate series of experiments showing that antianemic materials cause a marked reticulocytosis in mature male rats, although they admitted the test to be nonspecific.

Singer<sup>12</sup> found the rat to be suitable for the demonstration of the presence of the "Castle Factor" in human gastric juice. Normal human gastric juice caused a reticulocyte rise to two or three times the normal values in three to five days. The gastric contents of pernicious anemia patients in relapse had no such effect. These findings were partly confirmed by Hirschbacher and Schlesinger.<sup>7</sup>

Of the remaining laboratory animals, dogs and rabbits were tested for reticulocyte response to liver by Adlersberg and Gottsegen<sup>1</sup> who reported negative results. We believe that as yet a consistently reliable and practical method for assaying antipernicious anemia substances in experimental animals has not been established. Nor are we of the opinion that the presence of a megakaryoblastic bone marrow in some laboratory animals implies a pernicious anemia state. The changes in the bone marrow of a human pernicious anemia patient are secondary to a deficiency elsewhere in the body. Meeting this deficiency by appropriate therapy restores such bone marrow to normal but similar procedures in the test animal are productive of an abnormal condition for that animal. Hence we believe that the ideal laboratory assay for liver potency can be attained only by producing experimental pernicious anemia in an animal whose bone marrow normally resembles that of a normal human being, and this is our present objective.

#### SUMMARY

Thirty nine guinea pigs were studied for their response to antianemic materials. Liver extracts were given intraperitoneally and by mouth in sufficient doses to anticipate definite response. The results obtained and the normal unexplainable variations in reticulocyte counts permit the conclusion that in our hands the guinea pig was not a suitable animal for assaying antipernicious anemia potency of liver extracts.

A brief review of the literature on the use of other animals does not as yet conclusively demonstrate that any laboratory animal can supplant the human pernicious anemia patient for the assay of liver potency in the treatment of pernicious anemia.

#### REFERENCES

1. Adlersberg, D., and Gottsegen, G. Wirkung der Leberextrakte im Tierversuch, *Arch. f. exper. Path. u. Pharmacol.* 142: 323, 1929.
2. Castle, W. B., Townsend, W. C., and Heath, C. W. Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia, *Am. J. M. Sc.* 180: 305, 1930.
3. Clark, G. W., and Coe, A. M. Evaluation of the Antianemic Potency of Liver Extract by the Jacobson Method of Bioassay, *Proc. Am. Soc. Biol. Chemists*, Thirtieth Annual Meeting, p. 16, March 25, 1936.
4. Deutsch, W. Observations on Respiration of Red Blood Corpuscles of Normal Subjects and in Various Forms of Anemia, *Biochem. J.* 286: 2002, 1934.

a reticulocyte count of 22.2 per cent which gradually decreased to 0.8 per cent at the end of the third week, despite intraperitoneal injection of 2 gm. equivalent of fresh liver, which also failed to give a response in any of the 17 remaining animals.

TABLE I

GUINEA PIG	21	24	26
<i>Day</i>	<i>Control Period</i>		
1	2.0	0.6	
2	2.4	0.4	0.6
3	2.4	0.2	0.6
4	1.8	0.0	0.2
5	2.0	0.4	0.2
6	1.8	0.8	0.4
<i>Each pig injected intraperitoneally with the equivalent of 50 mg. of fresh liver</i>			
1	2.4	0.6	0.6
2	2.6	0.0	0.6
3	2.6	0.0	0.8
4	0.6	0.6	0.8
5	1.8	0.6	0.8
6	0.6	0.4	0.6
<i>Control Period</i>			
1	0.4	0.2	0.0
2	0.0	0.2	0.2
3	0.6	0.2	0.4
4	0.2	0.2	0.0
5	0.0	0.4	0.0
6	0.2	0.6	0.8
<i>Each pig injected intraperitoneally with the equivalent of 500 mg. of fresh liver</i>			
1	0.4	0.6	0.8
2	0.8	0.4	3.8
3	0.2	0.6	1.0
4	0.4	1.2	0.8
5	0.2	7.0	0.2
6	0.6	5.0	0.4
<i>Control Period</i>			
1	0.2		0.4
2	0.4	5.6	0.8
3	0.2	3.8	4.6
4	0.6	2.4	12.2
5	0.2	1.8	13.8
6	0.4	0.8	15.6
<i>Control Period</i>			
1	0.4	0.2	10.8
2	0.0	0.2	14.0
3	0.2	0.4	9.2
4	0.2	0.2	9.6
5	0.2	0.4	6.6
6	0.0	0.0	3.4
<i>Each pig injected with 2 c.c. of Chappel's Liver Extract. Two c.c. equivalent to 2 gm. fresh liver.</i>			
1	0.0	0.2	2.2
2	0.2	0.6	1.2
3	0.0	0.0	0.8
4	0.4	0.0	1.0
5	0.4	0.0	0.4
6	0.4	0.2	0.2

# LABORATORY METHODS

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## CHEMICAL DEMONSTRATION OF SMALL AMOUNTS OF BLOOD IN THE URINE\*

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SINCE Addis<sup>1</sup> in 1925 presented his method for the counting of the elements of urinary sediment, we have gained more accurate information concerning the blood content of the urine under normal and pathologic conditions. Thus Addis<sup>2</sup> found red blood cells in the urine in about two thirds of 74 normal persons, showing on an average 65,750 erythrocytes in the twelve hour night urine, with a maximum of 425,000. In a similar series of examinations from this department, Natar<sup>3</sup> found red blood cells in the urine in 51 out of 70 normal persons, the average being 130,000 erythrocytes in the twelve hour night urine, with a maximum of 1,100,000. Of particular significance to the present work are the studies carried out by Natar<sup>4</sup> on the amount of the hemorrhage in patients suffering from hemorrhagic Bright's disease. In the acute stage the twelve hour night urine contains as a rule from 2 000 millions to 8,000 millions red blood cells, and the maximum came as high as 10 000 millions which means about 2 cc of blood. When the number of erythrocytes in the twelve hour night urine falls off below 300 to 500 millions the condition of hematuria is no longer macroscopically visible. In the chronic stage of this disease the twelve hour night urine contains usually from 10 to 200 millions red blood cells, some patients excreting as a rule from 100 to 200 millions erythrocytes per night urine whereas others excrete only from 5 to 15 millions erythrocytes per night urine throughout long periods of their illness.

On the basis of these findings it looks interesting and promising to investigate whether the chemical reactions ordinarily employed in the clinic for demonstration of hematuria are sufficiently sensitive i.e., whether they can demonstrate a few millions of erythrocytes distributed in the twelve hour night urine. Among the many substances recommended for this purpose, e.g., *guaiac*, *aloin*, *phenolphthalein*, *fluorescein*, *benzidin*, and *orthotolidin*, only the three in italics, being typical representatives of the various substances, have been used for further examinations which comprise about 2,400 tests.

The guaiac test was presented in 1861 by van Deen<sup>5</sup>. It depends upon the ability of the hemoglobin to transfer the oxygen originating from turpentine or from hydrogen dioxide to the active elements of the gum guaiac, resulting in a blue compound that is soluble in neutral or acid solutions. Among the many

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TABLE II

GUINEA PIG	30	36	37	42
<i>Day</i>	<i>Control Period</i>			
1	0.2	22.2	0.8	0.4
2	0.0	16.8	0.0	0.6
3	0.2	9.8	0.6	0.0
4	0.0	7.0	0.6	0.2
5	0.0	6.2	0.0	0.0
6	0.2	4.2	0.2	0.0
<i>Equivalent of 2 gm. fresh liver intraperitoneally</i>				
1	0.0	4.2	0.4	0.4
2	0.0	4.2	0.0	0.4
3	0.0	3.6	0.0	0.6
4	0.0	3.4	0.0	0.0
5	0.4	3.0	0.4	0.2
6	0.2	3.0	0.4	0.4
<i>*Control Period</i>				
1	0.2	1.8	0.4	0.0
2	0.4	2.2	0.2	0.4
3	0.2	1.6	0.2	0.4
4	0.4	0.8	0.4	0.0
5	0.6	0.8	0.2	0.2
6				
7	0.0	0.0	0.4	0.2
8	0.2	0.0	0.2	0.6
9	0.4	0.2	0.0	1.4
10	0.0	0.8	0.8	1.2
11	0.0	0.4	0.8	0.0
12	0.0	0.4	0.4	1.0
13				
14	0.4	2.2	2.2	0.8
15	0.2	0.2	0.4	1.8
16	0.0	0.6	0.0	2.2
17	0.0	0.0	0.0	3.0
18	0.0	0.0	0.4	0.6
19	0.0	0.0	0.0	0.2
20	0.6		1.6	0.4
21	0.2	0.0	3.6	0.0
22	0.2	0.2	2.4	0.6
23	1.6	0.0	0.6	1.2
24	3.0	0.0	2.6	0.6
25	1.0	0.8	2.2	0.4
26	0.8	0.2	0.2	0.2
27	0.2	0.4	0.0	0.2
28				
29	0.6	0.2	0.2	0.2
30	0.8	0.4	0.2	0.2
31	0.6	0.4	0.6	0.4
32	0.6	0.6	0.2	0.6
33	1.8	0.4	0.4	0.4
34	0.2	0.2	0.4	1.6
35	0.6	0.0	0.4	0.4
36	0.2	0.2	0.0	0.4
37	0.2	0.0	0.0	0.2
<i>One half Cubic Centimeter Lilly's Extract Solution Intraperitoneally</i>				
1	1.0	0.2	0.0	0.0
2	0.4	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0
4	0.6	0.0	0.2	0.2
5	0.2	0.2		0.6
6	0.4	0.2	0.0	0.6

\*All animals rested for six weeks prior to this control period.

benzidin employed. Gregersen's modification of this test has been adopted widely for demonstration of occult blood in feces. In this form the test is made with an 0.5 per cent benzidin solution which is prepared freshly before each test by dissolving a powder of 25 mg. of benzidin + 0.2 gm.  $\text{BaO}_2$  in 5 cc. of 50 per cent acetic acid. Gregersen gives two methods for demonstration of hematuria: (1) 1 cc. of urine is added to 2 cc. of 0.50 per cent benzidin solution in a test tube, (2) 1 drop of urine is placed on a slide placed on a white background, and 2 to 4 drops of benzidin solution is added to the urine. By these methods I was able to demonstrate the presence of from 10 to 200 million erythrocytes, or more, in urine portions of 400 cc.

One property common to all the above mentioned methods is that the test is much more sensitive for the presence of blood in water than for blood in urine. Urine has a rather strongly inhibitory effect on the establishment of the reactions, and concentrated urines are more inhibitory in this respect than are urines of low specific gravities. In order to eliminate this inhibitory action of the urine, E. Boas<sup>1</sup> has suggested the following modification of the benzidin test. About 25 cc. of urine is filtered through an ordinary filter when all the urine has passed through, the benzidin solution is dropped upon the sediment (without unfolding the filter paper). In order to make the test more sensitive, the reagent is made stronger, only 3 to 4 cc. of 50 per cent acetic acid being added to the above mentioned amount of benzidin barium powder (giving a benzidin solution of 0.83 to 0.63 per cent). In this form the test is very sensitive, clear urines of low specific gravity give a positive reaction for the presence of even such a small number as 0.02 million erythrocytes in 10 cc. of urine, i.e., 0.8 million erythrocytes in 400 cc. of urine, that is a number of red blood cells occurring in the urines of normal persons.

On the basis of the method given by E. Boas I have elaborated a technique for demonstration of from 2 to 10 million erythrocytes or more in night urine of 150 to 700 cc. The reasons why I selected the Boas method are as follows. It is the only test available at present that has proved sufficiently sensitive, the test is easy to make and it takes but little time and only a few utensils, the reagents employed for this test keep relatively well. The test is made on the night urine, because our knowledge as to the degrees of hematuria is still limited largely to findings in the night urine. In addition, as will also be evident from the following, it is a somewhat significant point that the amount and specific gravity of the twelve hour night urine are usually subject to less variation than are the same features of the day urine.

The final establishment of the technique of this test met with several difficulties, however. One of these difficulties was due to the variability of the amount of the night urine. If, for instance, a test gives a negative result for 0.05 million erythrocytes in 10 cc. of urine and a positive reaction for 0.2 million erythrocytes, it means that with a twelve hour urinary output of 200 cc. it is practicable to demonstrate the presence of from 1 to 4 million erythrocytes or more in the total twelve hour urine. If, for example, the twelve hour night urine amounts to 600 cc., it is possible with this test to demonstrate the presence of from 3 to 13 million erythrocytes or more. A small twelve hour urinary output may thus give a positive reaction even though the number of erythrocytes

Vaughan, Muller, and Zetzel<sup>22</sup> and practically confirmed by Edmunds, Brueckner and Fritzell.<sup>6</sup> Wills<sup>23</sup> carefully repeated the work of Vaughan and her coworkers and found that the normal reticulocyte fluctuations were of such frequency and magnitude "that it was impossible to judge the response of any drug by the number present." "Obviously the variations in the percentage of reticulocytes in pigeons depend on other factors than those operating in man, and the greatest caution is therefore necessary in the interpretation of results."

Muller<sup>16</sup> however, in a study on the influence of liver extract and acute infection on reticulocytes and bone marrow of pigeons, concluded that the pigeon not only responded to liver extract with a rise in reticulocytes, but that infection had the same inhibiting influence on the response of pigeons to liver as it had on the response in human pernicious anemia patients. Peabody and Neale<sup>18</sup> found that the reticulocytosis in pigeons following injections of liver products did not parallel the clinical potency of the product. Heiman, Connery, and Goldwater<sup>19</sup> reported a marked daily variation in pigeon reticulocyte counts. This was unaltered by diet and the administration of liver extracts.

Gurd<sup>9</sup> reviewed the methods and results reported by the above groups, and concluded that none of the work was reliable, because the methods used failed to stain completely the reticulum of the pigeons' red cells. Using a more sensitive method he was unable to show any response to administration of potent liver extracts to the grain-fed pigeon and concluded that the pigeon was not a suitable means of liver assay.

Recently Muller<sup>17</sup> has found that liver extracts and nonspecific reticulogenic substances (intravenous injections of lysine and leucine) will be followed by "similar" reticulocyte responses in the pigeon under standard conditions. "These reticulocyte responses in the peripheral blood were accompanied by two histologically different reactions of the bone marrow. (a) After the administration of liver extract the megaloblasts of the bone marrow were transformed to more adult red blood cells. This occurs also in the bone marrow of pernicious anemia patients after liver therapy. (b) After the administration of lysine and leucine the bone marrow showed an increase and extension of erythroblastic tissue. Numerous megaloblasts and many mitotic figures were present." These observations do not, in our opinion, meet the criticisms advanced by Gurd nor establish the pigeon as an adequate assay animal.

A simple test tube reaction for the estimation of liver potency was described by Duesberg and Koll;<sup>5</sup> these investigators reported that when a suspension of washed erythrocytes was incubated with liver extract and subsequently hemolyzed, methemoglobin was formed in amounts proportional to the clinical activity of the liver. This observation was later contradicted by Deutsch,<sup>4</sup> and by Wilkinson and Deutsch.<sup>24</sup> They showed that the methemoglobin forming fraction could be separated by careful heating from the antianemic fraction, and that the methemoglobin-forming property of liver was independent of its clinical potency.

The American and German investigators disagree on the value of the rat as a hemopoietic test animal. Vaughan and Muller,<sup>23</sup> discoverers and proponents of the pigeon method of assay, reasoned that the rat would not be expected to



5. Duesberg, R., and Koll, W.: Über Methämoglobin bildung durch antianämisch wirkende organextrakte, Arch. f. exper. Path. u. Pharmacol. 162: 296, 1931.
6. Edmunds, C. W., Brueckner, H. H., and Fritzell, A. I.: A Laboratory Test for Liver Extract, J. Am. Pharm. A. 22: 91, 1933.
7. Fleischhacker, H., and Schlesinger, A.: Reticulozytenkrisen bei Ratten nach injektion von magensaft, Med. Klin. 31: 182, 1935.
8. Gebhardt, H., and Cario, R.: Die Wirkung von magensaft und einigen Verdauungsprodukten auf retikulozytenzahl und Blutregeneration, Med. Wchnschr. 58: 726, 1932.
9. Gurd, M. R.: Use of Grain-Fed Pigeons in Biological Assay of Liver Preparations, Quart. J. Pharm. & Pharmacol. 8: 39, 1935.
10. Heiman, H., Connery, J. E., and Goldwater, L. J.: Lack of Effect of Liver Treatment on Circulating Reticuloocytes in Pigeons, Am. J. M. Sc. 188: 343, 1934.
11. Jacobson, B. M.: Response of Guinea Pig's Reticuloocytes to Substances Effective in Pernicious Anemia. Biologic Assay of Therapeutic Potency of Liver Extracts, J. Clin. Investigation 14: 665, 1935.
12. Jacobson, B. M.: Assay on Guinea Pigs of Hematopoietic Activity of Human Livers, Normal and Pernicious Anemia, Ibid. 14: 679, 1935.
13. Klein, L., and Wilkinson, J. F.: Investigations on the Nature of Haemopoietin, the Anti-Anemic Substance in Hog's Stomach, Biochem. J. 27: 600, 1933.
14. Landsberg, J. W., and Thompson, M. R.: Guinea Pig as Hematopoietic Test Animal, Preliminary Report, J. Am. Pharm. A. 23: 964, 1934.
15. Miller, D. K., and Rhoads, C. P.: Reticuloocyte Response in Guinea Pigs Following Oral Administration of Certain Anti-Anemic Substances, New England J. Med. 213: 99, 1935.
16. Muller, G. L.: Influence of Liver Extract and Acute Infection on the Reticuloocytes and Bone Marrow of Pigeons, Arch. Path. 14: 774, 1932.
17. Muller, G. L.: Reticuloocyte Responses in Pigeon Produced by Material Effective and Noneffective in Pernicious Anemia With Description of Histologically Different Reactions of Bone Marrow, New England J. Med. 213: 1221, 1935.
18. Peabody, W. A., and Neale, R. C.: Pigeon as Hematopoietic Test Animal, J. Am. Pharm. A. 22: 1931, 1933.
19. Singer, K.: Über eine tierexperimentelle methode zum nachweis des Castle-Prinzips des magensaftes und klinische Bedeutung, Klin. Wchnschr. 14: 200, 1935.
20. Subbarow, Y., Jacobson, B. M., and Fiske, C. H.: Separation of Substances in Liver Which Are Reticulocytogenic in Guinea Pigs and Which Are Therapeutically Effective in Experimental Canine Black Tongue, New England J. Med. 212: 663, 1935.
21. Vaughan, J. M., Muller, G. L., and Minot, G. R.: Response Obtained in Healthy Pigeons by Administration of Substances Effective in Pernicious Anemia, Lancet 1: 1062, 1930.
22. Vaughan, J. M., Muller, G. L., and Zetzel, A. B.: Response of Grain-Fed Pigeons to Substances Effective in Pernicious Anemia, Exper. Path. 11: 456, 1930.
23. Vaughan, J. M., and Muller, G. L.: Effect of Liver and Commercial Liver Extract on Body Weight, Red Cells and Reticuloocytes of Normal Rats, J. Clin. Investigation 11: 129, 1932.
24. Wilkinson, J. L., and Deutsch, W.: Der "Methämoglobintest" für die Bestimmung der antianämischen Wirksamkeit von Leberextrakten, Klin. Wchnschr. 14: 926, 1935.
25. Wills, L.: Spontaneous Fluctuations in Reticuloocyte Count in Pigeon's Blood, Brit. J. Exper. Path. 13: 172, 1932.

to be present in such large amounts that their presence can never give rise to any error. It was found experimentally that 10 million yeast cells in 10 cc of urine (sp. gr. 1.015) gave a negative reaction, 4 million yeast cells in 10 cc of physiologic salt solution on the other hand gave a positive reaction and this was stronger after the yeast suspension had been boiled for two minutes. With the blood suspensions employed above the reaction was also found to be a little stronger after boiling for two minutes than before boiling. Urines giving a positive reaction for pus (jelling with alkali) will always give a positive benzidin reaction, and the reaction is not weaker after boiling for two to five minutes, this is due either to the possibility that pus cells behave in this respect like yeast cells and erythrocytes or, what appears to be the general view, that in any severe degree of pyuria there are always so many erythrocytes in the urine that they will give a positive reaction. The test is therefore, not applicable to urines that gelatinize with alkali.

The test may be performed in artificial light.

The benzidin reaction of the urine remains unchanged at least for one day if the urine remains clear. If it becomes turbid by precipitation of salts or by bacterial decomposition, the sensitivity of the test as already mentioned is lowered a little.

Further information about the degree of hematuria might be obtained by making the test with different weaker benzidin concentrations. Thus, for example, a positive reaction with a benzidin concentration giving a negative result for 1 million erythrocytes and a positive for 4 million erythrocytes in 10 cc of different urines, would indicate the presence of from 30 to 200 million erythrocytes or more in the total portion of urine. But just as valuable information is obtained with the test described above. As will be noticed from Table I, a reaction that appears within one, two, or three seconds (when the color will always be strong) will in most cases indicate that there are 1 million erythrocytes or more in 10 cc of the urine. As it makes no difference in the clinical estimation of a case whether there be 30 or 50 million erythrocytes in the night urine, this may be expressed as follows. A strong reaction within one to three seconds indicates the presence of about 50 million erythrocytes or more in the total portion.

All told, the method here described requires more preparatory measures than were previously considered necessary for a test for blood in the urine. Instruction of the patient about the collection of the urine voided in the night and early in the morning, in some cases with additional instruction not to take too much fluid, and in the case of a female patient, to wash the genitals thoroughly, measuring of the urinary output, determination of the specific gravity, and drawing off 5, 10, or 15 cc of urine. But this may all be done very quickly, once the examiner has become accustomed to it. The test itself is so easy to perform and requires so little apparatus, neither a centrifuge nor a microscope, that it also appears to be serviceable to the general practitioner.

While performing the studies here reported, I have had occasion to ascertain that Griegensen's benzidin barium powder is stand being kept in storage only for a certain length of time. When newly mixed, the powder is ivory colored, almost white, on storage it gradually becomes yellowish and finally brownish.

subsequent modifications of this method, the one given by Schroeder appears to be the best. Schroeder<sup>12</sup> has shown that the outcome of the reaction is dependent upon a certain relation between the amount of blood and the amount of guaiac, so that strong solutions of blood require strong solutions of guaiac, and weak solutions of blood require weak guaiac solutions, whereas the reaction may fail to appear if a strong guaiac solution is used together with a weak blood solution. As the blood content of the urine is unknown, the test is made with three different guaiac dilutions, as outlined in the following schema:

Tube 1: 10 drops tincture of guaiac\* + 2 c.c. of absolute alcohol + equal parts (i.e., about 2 c.c.) of turpentine.

Tube 2: 2 drops of guaiac tincture + 2 c.c. of absolute alcohol + about 2 c.c. of turpentine.

Tube 3: 2 drops from Tube 2 + 2 c.c. of abs. alcohol + about 2 c.c. of turpentine.

To each tube is added 10 c.c. of urine; then the mixture is shaken well. Using this test I found positive reactions for the presence of 120 million erythrocytes or more in 400 c.c. of clear urines of low specific gravity, whereas urines of higher specific gravities gave no positive reaction until the number of erythrocytes reached 400 millions or more. So this test is but little sensitive and also somewhat circumstantial.

The phenolphthalein test was presented first by Kastle and Shedd,<sup>9</sup> subsequently elaborated in particular by J. Boas,<sup>5</sup> and Johannessen.<sup>8</sup> It is based upon the fact that the colorless phenolphthalein in alkaline solution is readily oxidized by hydrogen peroxide, alcohol and blood to phenolphthalein, which gives a red color in alkaline solution. The reagent here employed is prepared as follows:

Phenolphthalein	1 gm.
Potassium hydroxide	25 gm.
Distilled water	100 gm.

are boiled together with 10 gm. of zinc powder to decoloration, then filtered. To this filtrate is added an equal amount of 96 per cent alcohol. The test is performed by mixing in a small test tube 0.5 c.c. of this alcoholic reagent, one drop of hydrogen peroxide and 0.5 c.c. of urine. With a twelve-hour urine portion amounting to 400 c.c., this test in my examinations gave a positive reaction for 12 to 20 million erythrocytes or more in the portion, if the urine was clear and of a low specific gravity. If the specific gravity was greater, the reaction was found positive only by the presence of 200 million erythrocytes or more. Besides the rather poor sensitivity of this test, the weak reactions are hard to recognize, as the faintly red colors make a much poorer contrast to the urine's own color than do the blue and green colors of the guaiac and benzidin reactions.

The benzidin reaction, first elaborated by O. and R. Adler,<sup>3</sup> is based on the fact that a solution of benzidin in acetic acid, containing hydrogen peroxide, turns greenish blue or blue when small amounts of hemoglobin are added. The sensitivity of the test depends to a large extent upon the concentration of

\*The preparation here employed is tincture of guaiac, 1:5, prepared by maceration of a not-too-old gum guaiac for about 1 month at 15° C. The finished tincture must be dark brownish red in color, it should be kept in a brown bottle.

A technic, based on the Boas test, is here given for demonstration of from 2 to 10 million erythrocytes or more in urine of 150 to 700 cc

## REFERENCES

- 1 Addis, Th J. A M A 85 163, 1925
- 2 Idem J Clin Investigation 2 409, 1926
- 3 Adler, O, and Adler, R Ztschr f Physiol Chem 41 11 1904
- 4 Boas, E Ugesk f læger 90. 1074, 1928
- 5 Boas, J Deutsche med Wchnschr, p 349, 1913
- 6 v Dean Quoted by Schroeder
- 7 Gregersen, J P Arch f Verdauungskr 25 169 1911
- 8 Johannessen, A Ugesk f læger 83 1613, 1921
- 9 Kastle and Shedd Quoted by Johannessen
- 10 Narna, A Hospitalstid 77 1445, 1934
- 11 Idem Ugesk f læger 97 531, 1935
- 12 Schroeder, K Hospitalstid 50 253, 1907

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## THE ESTIMATION OF CHOLESTEROL IN BLOOD\*

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### SUPPLEMENTARY NOTES ON A METHOD UTILIZING THE BERNOULLI REACTION

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**I**N 1933 we published a method for the estimation of cholesterol in blood,<sup>1</sup> utilizing the Tschugaew Bernoulli reaction. Since this date the technic has been in daily routine use in our laboratories. Over two thousand estimations have been carried out, and we have had ample opportunity for watching for any fallacies in the technic. Time has not caused us to make any modification in the method.

It is rather surprising to us therefore that the only published comments on our technic have been of an adverse nature. In 1934 Bloor<sup>2</sup> gave it as his opinion that "There is as yet no good reason for abandoning the colorimetric method in favor of digitonin precipitation." He believes that neither procedure is specific and the colorimetric method is easier to carry out. In criticizing digitonin precipitation he says, "It is not beyond possibility that the lower figures obtained with digitonin are due to hydrolysis of the digitonide, or incomplete precipitation as pointed out by Schonheimer and Dam."<sup>3</sup> In 1935 Artom<sup>4</sup> states, "The Bernoulli reaction is discredited." Ansbacher and Supplee<sup>5</sup> were unable to obtain consistent results with their modification of our technic applied to the estimation of cholesterol in milk.

In view of the consistently accurate results which our technic has given us, even in the hands of relatively unskilled laboratory technicians, it occurs to us that the possible explanation of the difficulty found by others in applying our technic lies in the fact that our original paper did not give sufficient details of the different stages of procedure. We consider that these supplementary notes might help other workers to overcome seeming difficulties.

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comes within the normal limit; on the other hand, if the twelve-hour urinary output is rather great, even absolutely pathologic values may escape demonstration. This difficulty is counterbalanced somewhat if the test is made with only 5 c.c. of urine when the total night urine amounts to less than 300 c.c., 10 c.c. when the total urinary output is between 300 and 500 c.c., and 15 c.c. when the total twelve-hour output exceeds 500 c.c. In this way a positive reaction indicates that the night urine contains, irrespective of its volume, from about 2 to about 10 million erythrocytes or more. The prerequisite of this tripartition is that the sensitivity of the test depends alone on the number of blood corpuscles present and is independent of the amount of urine passing through the filter; that this is actually the case to a sufficient degree was clearly demonstrated by performing the reaction on a series of urine samples of different volumes (5, 10, and 15 c.c.) and specific gravities, each sample containing the same number of red blood cells.

Another difficulty hinges on the fact that the sensitivity of the reaction differs in various urines depending upon the concentration and clearness of the urine. This is evident from the following experiment, which was performed in order to find, if possible, a benzidin concentration that would give in all urines a positive reaction for 0.2 million erythrocytes in 10 c.c. of urine and a negative reaction for 0.05 million erythrocytes in 10 c.c. of urine.

The night urines of several persons with healthy kidneys were mixed into portions of about 1,000 c.c. in such a way that the various portions differed in specific gravity. These mixtures were examined in the usual way for the presence of albumin, sugar, and pus, besides being examined microscopically. In addition, the erythrocytes were counted after the Addis method, and a red cell count in excess of 10,000 per 10 c.c. of urine was given consideration by preparation of the most diluted blood suspensions. A stock suspension containing 1 million erythrocytes per c.c. of urine was made for each mixture, 25 c.mm. of blood being added to 125 c.c. of urine. The blood used for these suspensions contained from 4.80 to 5.12 million erythrocytes per c.mm., and an amount of hemoglobin varying from 88 to 103 per cent (Sahli). By diluting different amounts of these stock solutions with up to 100 c.c. of urine, specimens of urine were prepared which contained from 5 to 0.02 million erythrocytes per 10 c.c. of urine.

The test itself was performed as follows: 10 c.c. of urine was pipetted off into an ordinary folded filter paper (diameter 10 cm.). After all the fluid had passed through the filter, the filter paper was spread out on a dry filter; the last remnant of fluid was absorbed by the dry filter, so that the original filter paper no longer shone from moisture. Then 6 or 8 drops of the reagent were placed in the center of the filter. This technic appears preferable to the Boas method, as a remnant of urine in the filter may inhibit the reaction. The reagent was prepared after the method given by Gregersen, except that the amount of benzidin was varied so as to make the various benzidin concentrations: 0.25 per cent, 0.30 per cent, 0.40 per cent, etc.

By such experiments it was found that a benzidin concentration of 0.50 per cent gave the most serviceable results. Table I shows the results obtained with this benzidin concentration in various mixtures of urines. As will be noticed, a

c That there is no optimum concentration of  $H_2SO_4$  for any given range of cholesterol concentrations—each concentration has its own optimum

d That the conditions of the reaction can be fixed only if the concentration of cholesterol is first known

These remarks refer to the pure cholesterol solutions. Using cholesterol ester solutions, the above remarks were also found to apply, but further, the optimum conditions for each concentration were different from those applying to free cholesterol.

These facts make the "direct" Liebermann Burchard reaction useless for measuring a mixture of cholesterol and cholesterol esters even in pure solutions.

Thus, a continued advocacy of the Liebermann Burchard reaction seems to us unjustified.

The observations of Gardner and Fox,<sup>7</sup> Lefschütz,<sup>8</sup> and Bohn and Brickenbach,<sup>9</sup> showed that bile pigments, lipochromes and resinous substances present in blood, all modify the color arrived at by the Liebermann Burchard reaction.

If practical conclusions are to be drawn from blood cholesterol estimations it is essential that the figures should be comparative and therefore determined by an accurate method. It is obvious therefore that any reliable technique must incorporate (a) the preliminary separation of cholesterol from interfering substances, which can only be done by preliminary separation with digitonin, and (b) the preliminary hydrolysis of cholesterol esters.

#### THE INDIRECT LIEBERMANN BURCHARD REACTION

*After Preliminary Separation of Cholesterol as Digitonide*—Schonheimer and Sperry,<sup>10</sup> have since published a technique indicating their view also that a separation of cholesterol from interfering substances was essential. They estimate the cholesterol on a digitonin precipitate with a modification of the Liebermann Burchard reaction. The digitonide is dissolved in acetic acid, and acetic anhydride and sulphuric acid are added.

In our experience, when this reaction is applied to cholesterol digitonide, the conditions governing color development are similar to those governing the pure cholesterol solutions. The possible variations are somewhat greater, however, in view of the fact that cholesterol digitonide gives a much weaker color than cholesterol.

Recently we attempted a comparison of Schonheimer and Sperry's technique with our own.

We were unable to arrive at consistently accurate figures when such a small amount of plasma was used. The chief difficulty seemed to be in the centrifug

TABLE II

SCHONHEIMER AND SPERRY (0.25 ML PLASMA)		OBERMER AND MILTON	
TOTAL	FREE	TOTAL	FREE
218	64	217	53
208	64	210	56
208	52	210	60
208	54	217	60
206	54	210	56
206		210	60
204		213	60
		213	

reagent with 0.50 per cent benzidin gives a positive reaction for normal amounts of erythrocytes in the less concentrated urines (sp. gr. less than 1.015). One will then have to omit the test on such urines or make it with a weaker benzidin concentration. I prefer the former alternative, as the specific gravity of the night urine will in most cases be 1.015 or more (or it may be made so by withholding fluids), and the erythrocytes keep better in a concentrated urine than in a thin urine.

Various factors that may contribute to the inhibitory effect of the urine upon the benzidin reaction are examined and discussed below. As a rule, almost without any exception, it can be said that when the urine is clear or only slightly cloudy, this inhibition increases with the specific gravity. If the urine is very turbid, the sensitivity of the test is decreased a little further, so that even the presence of 15 to 20 million erythrocytes in the total portion will in a few cases fail to give any reaction. Marked turbidity of the night urine is in a great many cases due to precipitation of urates. In such cases the test is performed after the urine has been made clear by heating slowly; then the sensitivity of the test, as has been demonstrated experimentally, is quite the same as in a clear urine. In the great majority of cases the night urine is acid; if, however, it is turbid and alkaline at the same time, its reaction has to be changed to acid before the test is made (by addition of acetic acid to dissolve phosphates and carbonates).

From the observation that the benzidin test was more sensitive after precipitation of the chlorides of the urine by means of silver nitrate, Johannessen assumed that the different inhibitory effect of the various urines upon the reaction was dependent upon the varying salt content. It seems more likely, however, that the increased sensitivity of the test after the aforementioned precipitation with silver nitrate is due rather to a clearing of the urine, as the voluminous precipitate of silver chloride takes along with it a great many of the formed elements of the urine. To throw some additional light on this question, I prepared some blood suspensions of equal strength in 0.9, 1.5, and 3 per cent NaCl solutions, respectively; the reaction was the same in all three instances. Then to the suspensions in 0.9 per cent NaCl solution were added the substances occurring most commonly in the urine, in such amounts as to approach the concentrations encountered in the urine. In such experiment no inhibitory effect was observed from phosphates, urea, creatinine, hippuric acid and oxalic acid. Urates, on the other hand, were found to inhibit the reaction, no matter whether they were precipitated or dissolved, but the latter finding could not be demonstrated in experiments with urine. Also tricresol and formalin inhibit the reaction, so that the test is not to be made on urines that are preserved by addition of these substances. A strong inhibition of the reaction was observed after addition of a thin suspension of talcum powder. These experiments suggest that it is the formed elements of the urine (bacteria, crystals, etc.) which have an inhibitory effect upon the benzidin reaction merely by their presence and independent of their chemical properties.

Other cells in the urine may give a positive benzidin reaction, e.g., yeast and pus cells. In order to give a positive reaction, however, the yeast cells have

The extract should be neutral in reaction before the digitonin is added. Acid tends to inhibit the coupling. The dry residue is heated with a little water to dissolve the digitonin. A fat emulsion forms, which dissolves when about three times the volume of acetone is added.

The suspension is transferred to a centrifuge tube. The nature of the fluid, which contains fats and excess digitonin, makes packing of the precipitate very difficult.

In our experience it is here that gross errors may creep in. Very intensive centrifuging is necessary to throw down the precipitate and even then the removal of the supernatant fluid should be carried out with the greatest care in order to prevent any of the precipitate from being sucked off.

In following the technique of Schonheimer and Speers (where only minute quantities of precipitate are being dealt with) we found that errors up to 100 per cent could be accounted for in this way.

The device of adding  $\text{AlCl}_3$  and precipitating  $\text{Al}(\text{OH})_3$  from solution with ammonia is useful in entraining the digitonide. On centrifuging packing of the precipitate then occurs quite readily.

The aluminum hydrate is dissolved out with a drop of acid in subsequent washing, as centrifuging difficulties are not encountered once the bulk of the fat and excess digitonin has been removed.

*Hydrolysis of Cholesterol Esters*—Sodium ethylate is the most effective hydrolytic agent. Small amounts should be prepared frequently from sodium and absolute alcohol, and discarded when they show a brownish tinge.

Hydrolysis of cholesterol esters is complete on boiling for less than thirty minutes. In order that fatty substances which cause centrifuging difficulties be hydrolyzed, boiling is, however, prolonged for this full period.

The bulk of the solvent should then be distilled off. On no account should the contents of the flask be allowed to dry. If this occurs, the estimation must be restarted. Apparently the cholesterol is so altered by the action of the soda as to prevent coupling with the digitonin.

When the contents of the flask have been reduced to about 2 ml, about 20 ml of petroleum ether are added, and the mixture brought to a boil. Two milliliters of water are added, and the flask thoroughly shaken. This dissolves out the soda, alcohol, and any coloration produced during hydrolysis.

The flask is allowed to stand until the water layer has separated to the bottom. The petroleum ether layer is decanted off through a filter paper. The water layer is extracted with a further quantity of petroleum ether. The petroleum fractions are mixed, alcoholic digitonin added, and the contents evaporated to small bulk. About 5 ml of alcohol are added, and the contents evaporated to dryness.

The centrifuging and washing of the precipitate are the same as for free cholesterol.

Occasionally, despite all precaution, minute quantities of soda find their way into the petroleum extract. This has the effect of causing delayed precipitation of the digitonide. It is, therefore, advisable to luxivate the digitonide with 1 per cent hydrochloric acid rather than with water, at the centrifuging stage.

yellow, and its sensitivity decreases with this change in color. The color changes more slowly if the powder is kept in black paper capsules than when it is kept in white capsules. In one experiment with a benzidin powder that was 13 months old and had turned brown in color, it was possible in a urine with a specific gravity of 1.018 to demonstrate 0.5 million erythrocytes per 10 c.c. of urine, but not 0.3 and 0.2 million erythrocytes per 10 c.c. of urine, whereas the two last-mentioned values could be demonstrated with benzidin powders that were one week, one month, and two months old, respectively. So, benzidin powder that has turned brown is no longer fit for use.

In conclusion, I shall briefly recapitulate the technic of the test here suggested as it may be put down, for instance, in a printed list of directions:

The test is performed on the night urines with a specific gravity of 1.015 or more.

1. Determination of the specific gravity and amount of the night urine.
2. The entire portion of the night urine is shaken well.
3. If the total night urine amounts to less than 300 c.c., only 5 c.c. (measured by graduate or pipette) is filtered through ordinary filter paper; if the total night urine exceeds 300 c.c., but is less than 500 c.c., 10 c.c. is filtered; and if it exceeds 500 c.c., 15 c.c. is filtered.
4. The benzidin reagent is prepared by dissolving a Gregersen powder (25 mg. benzidin + 0.2 gm.  $\text{BaO}_2$ ) in 5 c.c. of 50 per cent acetic acid.
5. When all the urine has passed through the filter, the filter paper is spread out on a dry filter paper, and when the first filter has lost its shine of moisture, 5 or 6 drops of the benzidin reagent are placed in the middle of the filter.
6. If a blue color appears within one to three seconds, the strength of the reaction is designated by ++, which signifies that there are about 50 million erythrocytes or more in the total night urine. If it takes longer (from four to thirty seconds) the strength of the reaction is designated by +, which indicates the presence of from 5 to about 50 million erythrocytes in the night urine. A blue coloring that appears later than thirty seconds after the addition of the reagent is considered a negative reaction.
7. If the urine is very cloudy, and does not become clear on slow heating or on addition of acetic acid until it gives a distinctly acid reaction, the test is a little less sensitive.
8. The test is not made if the urine gelatinates on addition of alkali.

If the test is made on urine with a specific gravity of less than 1.015, a negative reaction shows, of course, that the amount of red blood corpuscles in the urine is not increased to pathologic values; if the reaction is positive, the test must be repeated on a urine of higher specific gravity.

#### SUMMARY

Schroeder's guaiac test, Johannessen's phenolphthalein test and Gregersen's benzidin test are not sensitive enough for demonstration of slight degrees of hematuria, whereas the E. Boas modification of the benzidin test will in several cases give a positive reaction even with a normal amount of erythrocytes in the urine.

- 6 Obermer, E, and Milton, R The Vernes Bricq Von Photometer—Its Application to Routine Biochemical Work, With Special Reference to the Estimation of Phosphorus in Blood, *J LAB & CLIN MED* 17 792, 1922
- 7 Gardner, A J, and Fox, F W A Critical Study of the Methods of Estimating Cholesterol and Its Esters in Tissues Part II *Biochem J* 18 1038 1934
- 8 Lifschultz, J Ueber die Oxydation des Cholesterins *Zt hr f Physiol Chem* 50 436 1906
- 9 Bohn, H, and Bickenbach, D Die Cholesterinbestimmung nach Autenrieth Funk ausgeführt mit dem Pulfrich'schen Streuphotometer *Ztchr f Exper Med* 71 566, 1930
- 10 Schonheimer, R, and Sperry, W M A Micro method for the Estimation of Free and Combined Cholesterol, *J Biol Chem* 108 747 1934

## RAPID METHODS FOR PREPARING AND STAINING BONE MARROW\*

L M SCHLICHTR, A B, AND E A SHARP, M D DETROIT, MICH

THERE is increasing appreciation of the diagnostic and prognostic value of hematologic data derived from a study of the bone marrow. The technical procedure of biopsy consists of two major steps: (1) The removal of bone marrow, and (2) preparation of the bone marrow for microscopic examination.

Various instruments have been developed for the operative procedure, all of them having their advantages and disadvantages. Similarly numerous methods for treating and staining marrow tissue have been described. Experience with various techniques, however, convinced us that none was entirely satisfactory since the primary objective—clear cut cytologic differentiation, was seldom attained.

It is the purpose of this report to present (1) a simple technique for preparing fresh bone marrow which permits individual and differential cytologic study, and (2) a rapid and efficient method for staining fresh marrow with May Grunwald and Giemsa stains.

*Technic for Bone Marrow Biopsy*—The operative technique for bone marrow biopsy described recently by Young and Osgood<sup>1</sup> has been used. Sufficient bone marrow for comprehensive study can be obtained in this manner. The type of needle devised by Khma and Rosegger,<sup>2</sup> Fig 1, is preferred to an 18 gauge spinal puncture needle, since it permits the operator to regulate the depth of the puncture in an accurate manner. A suitable adapter must be provided for this needle when using a syringe of domestic manufacture.

The site of puncture is the sternomanubrial junction. After the needle is inserted, the piston of the syringe (5 cc capacity) is partially withdrawn and held firmly until marrow appears. The maneuver should be rapid, since this will prevent admixture of peripheral blood. About 0.5 cc of marrow is withdrawn and discharged without delay into a small paraffin lined test tube, containing a sufficient amount of heparin to prevent coagulation. The tube is gently inverted several times to obtain an even mixture of the cellular elements.

A Wintrobe hematocrit tube is then filled with the heparinized marrow to the "10" mark and centrifuged at high speed for five minutes. After centrifuga-

\*From the Anemia Laboratory, Out Patient Department, Harper Hospital.  
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## THE "DIRECT" LIEBERMANN-BURCHARD REACTION

Our investigations prior to the publication of our method, carried out over a lengthy period, prove conclusively that the "direct" Liebermann-Burchard reaction is inapplicable to the analysis of cholesterol in blood.

Table I shows the amount of color development given with the Liebermann-Burchard reaction by varying amounts of cholesterol, under varying conditions of time, temperature, and amounts of sulphuric acid.

TABLE I  
DEVELOPMENT RATES WITH PURE CHOLESTEROL

0.1 ml. of solutions containing varying concentrations of cholesterol are made up to 5 ml. with chloroform.

2 ml. of acetic anhydride are added, and varying amounts of concentrated sulphuric acid. Development is allowed to proceed at given temperatures, and the optical densities of the color produced read at intervals on the Vernes-Brieq-Yvon Photometer<sup>6</sup>

0.05 ml. H <sub>2</sub> SO <sub>4</sub> used																					
MG. % CHOLESTEROL		DEVELOPMENT AT 0° C.							20° C.							37° C.					
		TIME IN MINUTES																			
		10	20	30	40	50	60	70	10	20	30	40	50	60	70	10	20	30	40	50	
80		6	10	12	14	15	16	16	10	11	12	11	11	--	--	14	15	13	12	11	
160		14	19	22	24	26	29	29	18	23	25	22	22	--	--	20	24	24	21	20	
240		16	24	30	34	36	35	34	30	34	33	32	31	--	--	30	33	32	31	29	
320		18	30	40	48	48	47	44	35	46	44	43	42	--	--	45	44	44	43	41	
400		18	30	38	48	58	56	52	46	54	53	52	51	--	--	50	57	54	52	50	
0.10 ml. H <sub>2</sub> SO <sub>4</sub> used																					
MG. % CHOLESTEROL		DEVELOPMENT AT 0° C.]							20° C.							37° C.					
		TIME IN MINUTES																			
			10	25	40	50	70	10	20	30	40	50	60	70	10	20	30	40	50		
80		13	16	15	13	12	14	13	12	11	11	--	--	16	14	11	10	10			
160		25	26	26	25	23	25	24	23	21	20	--	--	27	26	21	19	18			
240		18	43	48	46	44	30	34	34	32	30	--	--	40	34	32	30	25			
320		50	56	52	50	46	48	45	41	39	37	--	--	50	49	44	40	38			
400		64	64	60	57	54	60	58	52	50	46	--	--	60	57	50	46	45			
0.15 ml. H <sub>2</sub> SO <sub>4</sub> used																					
MG. % CHOLESTEROL		DEVELOPMENT AT 0° C.]							20° C.							37° C.					
		TIME IN MINUTES																			
			10	25	40	50	70	10	20	30	40	50	60	70	10	20	30	40	50		
80		14	16	16	14	14	10	12	12	10	10	--	--	20	15	14	11	11			
160		24	25	23	22	21	22	21	20	16	15	--	--	27	19	18	17	16			
240		36	34	32	32	31	35	33	27	22	21	--	--	38	31	26	25	25			
320		50	48	44	43	38	48	41	37	34	30	--	--	46	39	34	31	30			
400		66	60	56	52	51	55	50	45	40	37	--	--	58	50	43	40	37			
0.20 ml. H <sub>2</sub> SO <sub>4</sub> used																					
MG. % CHOLESTEROL		DEVELOPMENT AT 0° C.]							20° C.							37° C.					
		TIME IN MINUTES																			
			10	25	40	50	70	10	20	30	40	50	60	70	10	20	30	40	50		
80		14	12	12	10	10	12	9	9	8	7	--	--	11	11	10	10	9			
160		28	27	26	23	21	21	18	17	16	16	--	--	22	19	16	15	15			
240		40	36	36	34	32	32	27	24	23	22	--	--	34	29	25	24	23			
320		40	51	56	52	50	41	36	32	27	28	--	--	37	34	33	33	32			
400		53	66	67	63	32	50	42	41	37	32	--	--	52	45	39	38	36			

These figures permit of the following definite conclusions:

- That the peak of color development is transient, and that fading occurs.
- For each given temperature the time for maximum color development is different for varying concentrations of cholesterol.

slide is then removed and placed on a staining rack covered with 3 c.c. of dilute Giemsa solution (Giemsa stock solution, one drop, plus buffer solution pH 6.8, one cubic centimeter) for five minutes. (4) Finally rinse with distilled neutral water until the water returns clear while tilting the slide, then place in a vertical position and allow to air-dry.

If this procedure is followed, there will be obtained not only a clear but also a well-tinted smear. Several hundred comparative studies showed no appreciable difference between the cellular morphology in the bone marrow preparations and those from peripheral blood when both were stained by the above described technic.

A bone marrow microslide preparation of an acute hemolytic anemia is cited in Fig. 2 as a typical example of the brilliant preparations obtainable with this method. These bone marrow preparations have been successfully reproduced in color by photomicrography.

#### SUMMARY

1. Bone marrow is removed from the sternomanubrial junction.
2. A needle devised by Klima and Rosegger permits accurate adjustment of the depth of the puncture.
3. Erythrocytes and leucocytes in a ratio of 1:1 has been adopted as an arbitrary standard.
4. A rapid method of staining fresh marrow tissue with May-Grünwald and Giemsa stains at a pH of 6.8 has been described.
5. The clarity of cellular morphology of bone marrow, when treated as described herein, is comparable to that found in peripheral blood preparations.

#### REFERENCES

1. Young, R. H., and Osgood, E. E.: Sternal Marrow Aspirated During Life, *Arch. Int. Med.* 55: 187, 1935.
2. Klima, R., and Rosegger, H.: Zur Methodik der diagnostischen Sternalpunktion, *Klin. Wchnschr.* 13: 541, 1935.

ing and washing of the precipitate. It was almost impossible to avoid some washing loss, which, with such a small precipitate, caused significant errors.

Later we repeated the technic using five times the amount of plasma specified by the authors, i.e., 0.25 ml.

With this slight modification of the Schönheimer and Sperry technic, it will be seen that our results are closely comparative.

We would suggest, however, that the technical difficulties of the separation and the extreme care needed in standardizing the color development, render the technic less suitable for routine purposes than our own.

#### THE TSCHUGAEW-BERNOULLI REACTION

The disadvantages of the Liebermann-Burchard reaction led us to investigate the Tschugaew-Bernoulli reaction some time before we arrived at our final technic.

If an acyl chloride be heated with cholesterol in the presence of a dehydrating reagent, a reddish brown color is produced. If other conditions are constant, this color is proportional to the concentration of cholesterol present.

In practice we found ortho-nitrobenzoyl chloride to be the most satisfactory for the purpose. To produce maximum color intensity, boiling for at least fifty minutes is necessary.

If acetyl chloride is used, it tends to distil away. Thus a variable lowering of the concentration of one of the reacting solutions may lead to inconstant results.

Benzoyl chloride, if freshly distilled, will give a good color. On cooling, however, benzoic acid precipitates out, causing a cloudiness of the solution, which renders it unfit for color comparison. This also occurs with phenyl acetyl chloride, and para- and meta-nitro benzoyl chlorides. The ortho-nitro derivative, however, gives a very soluble acid and a clear solution is obtained. The latter has an added advantage of being solid at ordinary temperature, and is consequently more stable.

Schönheimer and Sperry criticized the reaction on the ground that the resultant color is the summation of color given by the cholesterol and digitonin. We regard this as an advantage, since a greater depth of color is obtained for a given amount of cholesterol present. This allows smaller variations in cholesterol content to be estimated with greater precision. It is true that excess digitonin must be washed away. This does not involve extra work, since a thorough washing of the cholesterol digitonide precipitate is necessary in any case.

*Separation of Cholesterol.*—We think it probable that some difficulty is encountered with the details of cholesterol digitonide precipitation.

The cholesterol is separated from blood by extraction with some solvent which also acts as a protein precipitant; 3:1 alcohol-ether, or 1:1 alcohol-acetone, is equally good. Alcoholic digitonin\* is added, and the mixture evaporated to dryness on a steam bath.

\*The digitonin solution is prepared as follows: 1 gm. of digitonin is dissolved in 50 ml. absolute alcohol. 50 ml. of water are added and the solution allowed to stand in ice overnight. Any precipitate which forms is filtered off.

being marked off in grams (0 to 1,000 with eight subdivisions—0, 125, 250, etc.). When the plunger is inserted into the heart muscle (interventricular septum), the spring within the tubes becomes compressed, and the graduated rod is pushed outward. After the plunger has pierced completely the cardiac muscle, the spring recoils, but the graduated rod remains in position, showing the number of grams required to perforate that cardiac area. In the heart, the center of the interventricular septum has been chosen because this area is least likely to show local changes due to coronary disease. The test is either performed with the excised interventricular septum or the interventricular septum in situ.

In a series of over 200 autopsies, I have found in normal hearts the reading with this instrument to be 1,000 to 1,050 gm. while degenerated hearts showed various readings down to 200 gm. The lower the reading, the more

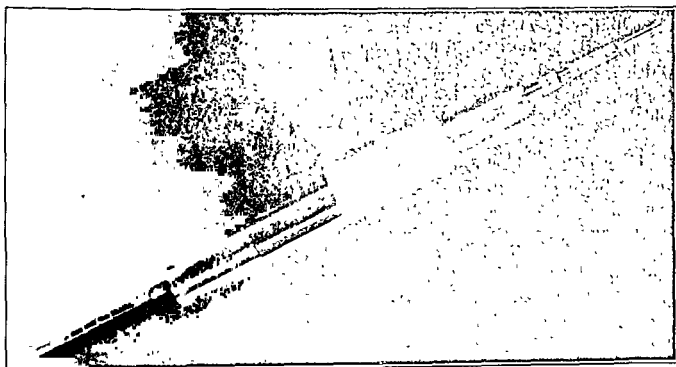


Fig. 2.—After test. In window region to right, note new reading (in grams pressure).

degenerated the cardiac muscle; whereas the closer the reading to 1,000, the less degenerated was the cardiac muscle. These gross findings were corroborated by histologic examination of the cardiac muscle.

#### SUMMARY

A new instrument is introduced as an additional measure to determine condition of cardiac muscle and degree of degeneration at autopsy.

Pressure fragmentation and hence myocardial degeneration can be expressed in terms of percentage, normal being 1,000.

Further studies will be necessary to classify these cases into markedly degenerated hearts, moderately degenerated, slightly degenerated, etc.

#### REFERENCES

1. Boyd: Text Book of Pathology, p. 356.
2. Sajous: Encyclopedia 5: p. 294.

*Details of Color Reaction.*—The digitonide is finally washed with ether and dried. It must be perfectly dry before proceeding to the color development, since water inhibits the reaction. Exclusion of water is the main precaution to observe during this stage of the procedure. All pipettes should be dry, and the tube should be covered when placed in the water-bath, to avoid entry of steam. The most effective device for covering the centrifuge tube is to use a capillated tube held in position with a rubber sleeve.

Zinc chloride, which is used as a condensing agent, must be anhydrous. This is obtained by fusing stick zinc chloride until fumes no longer arise, and dissolving the cooled melt in glacial acetic acid. The solution should be stored in a tightly stoppered bottle. We find the best practice is to add the 0.2 ml. of ortho-nitro benzoyl chloride last from the pipette, and not in solution of glacial acetic acid as originally indicated.

The time of color development in the boiling water-bath is fifty minutes. A very slight increase of color is obtained with all concentrations if the heating is continued for a longer period. The reaction is stopped, however, as soon as the tube is removed from the bath, and no difference in color is obtained if the tube is left at room temperature, even after twenty-four hours.

We have already referred to the paper of Ansbacher and Supplee on the estimation of cholesterol in milk.<sup>5</sup> They experienced difficulties when they attempted a modification of our technic.

They first tried para-nitro benzoyl chloride with unsatisfactory results, which were eliminated when benzoyl chloride was used. We emphasize the fact that no difficulties should be encountered with the ortho-nitro benzoyl compound, but that benzoyl chloride is likely to cause turbidity, which interferes with color comparison.

#### CONCLUSIONS

1. The "direct" Liebermann-Burchard reaction is unsuitable for accurate cholesterol estimation in blood.

2. With the microtechnic of Schönheimer and Sperry, utilizing the indirect Liebermann-Burchard reaction, good results can be arrived at, providing that (a) extreme care is taken with the conditions of color development, and (b) sufficient plasma is used; the small quantity of plasma used in their original technic can lead to gross errors owing to mechanical loss.

3. Reports which have appeared in the literature regarding the inaccuracy of the Bernoulli reaction as used in the Obermer-Milton technic, probably arise from the fact that insufficient details were published in the original article.

#### REFERENCES

1. Obermer, E., and Milton, R.: A Micro-Photometric Method for the Determination of Free Cholesterol and Cholesterol Esters in Blood Plasma, *Biochem. J.* 27: 345, 1933.
2. Bloor, W. R.: *Ann. Rev. Biochem.*, p. 175, 1934.
3. Schönheimer, R., and Dam, H.: Ueber die Spaltbarkeit und Löslichkeit von Sterin-digitoniden, *Ztschr. f. Physiol. Chem.* 215: 59, 1933.
4. Artom, C.: *Ann. Rev. Biochem.*, p. 199, 1935.
5. Ansbacher, S., and Supplee, G. C.: The Cholesterol Content and the Antirachitic Activation of Milk Constituents, *J. Biol. Chem.* 105: 291, 1934.

All metallic parts are brass, nickel plated except the spring, which is  $\frac{1}{16}$  inch in diameter, music or spring wire formed, but not retempered

The cotton or sponge is quickly and efficiently moistened with alcohol or other antiseptic and the exact amount desired to be delivered can be prede-

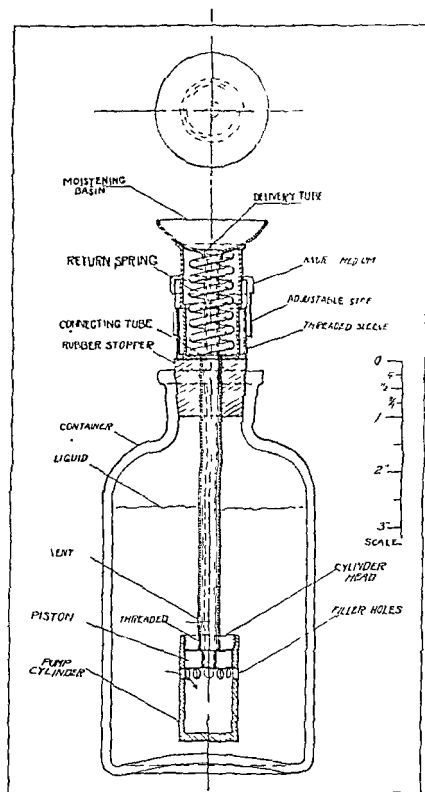


Fig. 1

Fig. 1.—Scale drawing of the apparatus.

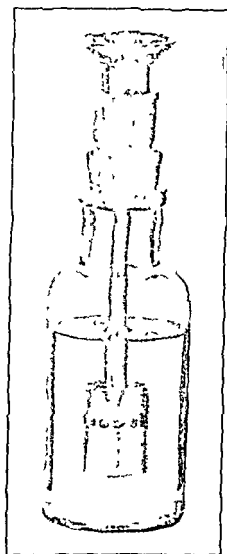


Fig. 2

Fig. 2.—Photograph of the apparatus.

termined and maintained. The use of the apparatus has facilitated the rapid bleeding and skin testing of many patients and has reduced to a great extent the volume of alcohol used. One of these instruments has been in constant use for eleven years and two others for seven years each; the only repairs have been replacement of the spring of each one.

tion the height of the white myeloid layer in the hematocrit is determined and an equal column of red cells is marked on the tube. The supernatant plasma is drawn off with a pipette until the plasma column is equal to the combined myeloid and erythrocyte layers. With a pipette, the plasma, myeloid, and erythrocyte layers are withdrawn, discharged into a paraffin-lined watch glass, and thoroughly mixed. A small drop is transferred to a slightly warm microslide, and by the use of a cover slip, a margin-free smear is made, as recommended by Schilling for peripheral blood. After the marrow preparation has been air-dried, it is ready for staining.

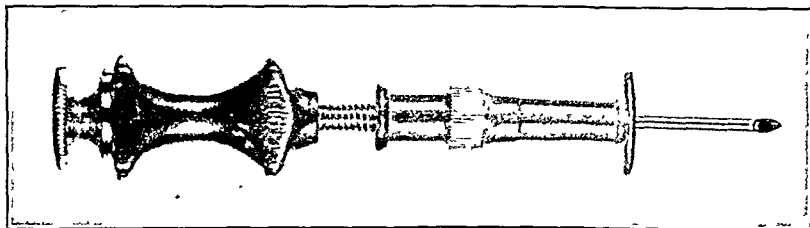


Fig. 1.—Klinta and Rosegger needle for bone marrow biopsy.



Fig. 2.—Bone marrow from sternum prepared and stained by the authors' methods. Preparation taken from an acute hemolytic anemia. The myelocytic granules and mitosis and structural characteristics of the megaloblast are particularly well illustrated. Magnification  $\times 1500$ .

**Rapid Staining of Bone Marrow.**—The slide is placed into (1) 0.3 per cent alcoholic May-Grünwald solution for three minutes. (2) Without washing, place slide into a freshly made-up dilute May-Grünwald solution\* for one minute (May-Grünwald solution and buffer solution pH 6.8, equal parts). (3) The

\*One hundred cubic centimeters of absolute methyl alcohol (National Aniline and Chemical Company, New York) are warmed to  $50^{\circ}\text{C}$ . To the warm alcohol is added, in minute portions, 0.3 gm. of May-Grünwald dye powder (National Aniline and Chemical Company, New York). After each addition of the dye the solution is shaken vigorously. When solution is complete, allow to stand for twenty-four hours, filter into a brown or blue bottle and store in a cool place. The dilute solution used in staining should be replaced daily. (In hot weather the diluted stain may deteriorate in two to three hours.) It is very important to keep the dye and buffer solutions cool.

summation method requires much more work than its estimation from the total fatty acid total cholesterol titration. The summation method, however, provides at the same time the individual lipid values.

The method used to obtain the conversion factor was as follows. The total lipid content of 100 cc of plasma was found by summation of individually estimated values. The total fatty acid total cholesterol titration was couched in terms of cubic centimeters of 0.1 N potassium dichromate and the value of this per 100 cc of plasma calculated from the aliquot used and the dilution of the original extract. The resulting titration figure was divided by the previously found total lipid value thus giving the factor by which the total fatty acid total cholesterol titration required to be divided to produce the total lipid.

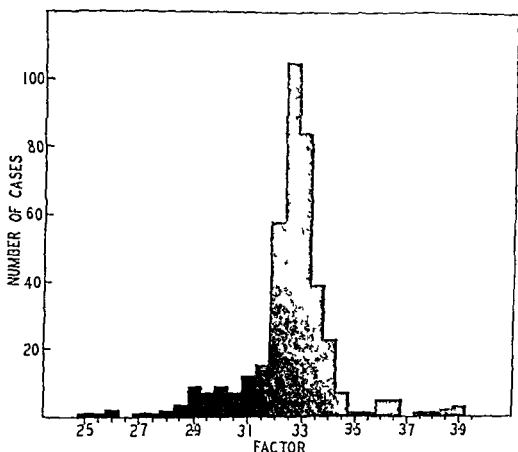


Fig 1—The frequency distribution of 406 experimentally determined values for converting a total fatty acid total cholesterol titration into total lipid of plasma

When this work was originally undertaken, it was anticipated that there would be so much variation in the factor that this simple method of *approximating* the total lipid of plasma would be subject to too much error. It was found, however, that the great majority of the determined factor values fell within a comparatively narrow range. Factor values between 3.1 and 3.4 were found in 336 of the 406 cases or 83 per cent in which the variation in the factor, and hence in the total lipid value calculated therefrom, was less than 10 per cent. The mean factor value of the 406 cases was 3.25 and the standard deviation (calculated as by Boyd<sup>3</sup>) was 0.15. The frequency distribution of the experimentally determined factor values is shown in Fig 1.

It is thus obvious that by use of the Bloo<sup>1</sup> technique<sup>2</sup> and of the conversion factor 3.25 a value for the plasma total lipid is obtained which is sufficiently

## A NEW INSTRUMENT FOR DETERMINING CARDIAC CONDITION AT AUTOPSY

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WHAT is clinically called myocardial degeneration may be one of a number of pathologic conditions, viz., parenchymatous degeneration, fatty degeneration, etc. Most cardiac degenerations come under the term parenchymatous degeneration, but yet there are various degrees of this. The pathologist, in examining a heart for pathologic changes, notes size, color, consistency, etc. If it is paler than normal, cloudy and half cooked in appearance, cuts with decreased resistance, and is softer than normal, it is called parenchymatous degeneration. The myocardial fiber is a specialized cell and is therefore subject to the usual degenerative changes from which cells suffer.<sup>1</sup> In general fatty degeneration, the muscular substance throughout presents a pale or a light yellowish appearance, and is quite friable, the finger being readily thrust into it.<sup>2</sup>

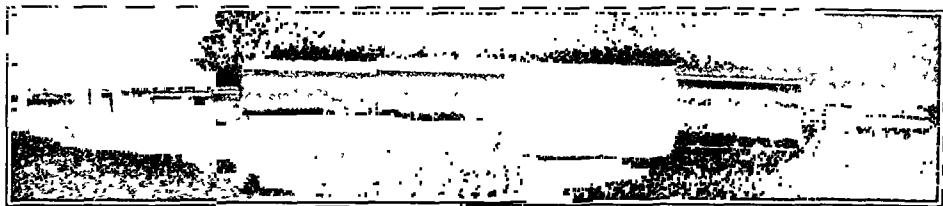


Fig. 1.—Before test. Number in the window region to right reads 0. Note serrated surface in middle of tube to insure firmer grip during use. Plunger is at left graduated into centimeters and half centimeters.

Therefore, degenerated cardiac muscle is more easily fragmented than normal cardiac muscle. A suitable term for this would be pressure fragmentation.†

A general idea of the amount of pressure required to perforate cardiac muscle can be gotten by pushing the index finger or thumb through the muscle. The more easily penetrated by the finger, the more degenerated the heart muscle. A more accurate test can be obtained by this instrument.

The instrument is made of brass, heavily chrome plated, and rust proof. There are two brass cylinders, one telescoping within the other. Inside is a brass spring, tempered to a fixed hardness. At the distal end is a plunger,  $\frac{1}{4}$  cm. long by  $6\frac{1}{4}$  mm. wide (diameter), graduated into centimeters and half centimeters. Inside of the tube is a brass graduated rod that projects from the proximal end of the instrument, the rod having a flattened surface and

\*From the Department of Pathology, Temple University School of Medicine and Jewish Hospital.

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†For this term I am indebted to Dr. Robinson, of Philadelphia General Hospital.

3 Long Wright's tubes with capillary tips. The body of the tube should be about 6 cm long and the internal diameter 0.4 to 0.45 cm. The tubing is first carefully cleaned with sulphuric bichromate solution rinsed thoroughly in tap water and finally with distilled water and dry sterilized before being drawn into Wright's tubes.

4 Alcohol lamp or other source of flame. Methenamine (urotropin) tablets give a hot, smokeless flame.

5 Sealing wax.

6 Corked test tubes and labels.

With these materials at hand moisten the finger of the adult or the ball of the great toe of the infant with alcohol and prick the skin fairly deeply in one or two places with the *sharp V shaped blade* so as to induce the flow of blood on slight pressure\*. Wipe off the blood from the site of the puncture with a sterile dry sponge and, holding the Wright's tube horizontally or at a slight inclination, apply one tip to the puncture, blood droplets, and the blood will enter the tube by capillary attraction. Care should be taken not to allow the column of blood to break and run down one side of the tube since this prevents the egress of air from the other tip and no more blood will enter. Fill the tube about three-fourths full. Warm the unfilled portion of the tube in the flame so as to expand the contained air, seal the tip in a flame, cool in sealing wax or air, shake or tap the blood down into the sealed end and seal the original tip in a flame or with a generous cap of sealing wax. Avoid overheating any part of the blood except that unavoidably charred in the tip. Label and place in a test tube container.

In the laboratory, the following materials are employed:

- 1 Small test tube, approximately 0.7 cm by 5.0 cm and racks
- 2 Pipettes graduated every 0.05 cc up to 0.2 cc with capillary tips
- 3 Ordinary 1 cc pipettes with 0.1 cc graduations
- 4 Ordinary 5 cc pipettes with 0.5 cc graduations
- 5 Alcohol lamp or other flame
- 6 Sealing wax
- 7 Triangular file
- 8 Microburner, with or without oxygen, or ordinary Bunsen burner
- 9 Microscope
- 10 36° and 37° C water baths
- 11 Centrifuge
- 12 Centrifuge tubes with cotton packed into the tips
- 13 Regular Hinton indicator
- 14 Physiologic saline solution
- 15 Rubber tubing with mouthpiece, such as used with blood counting pipettes

Having ascertained that the Wright's tube containing the blood specimen is well sealed at the tip, this tip is placed downward in a numbered centrifuge tube and centrifuged at high speed for ten minutes. Should the serum not separate well in this length of time file the tube, break it open, and loosen the clot with a toothpick or wire. Recentrifuging will then separate the serum.

\*In the case of the infant the flow of blood from the toe can be improved by preliminary massage by immersion of the foot in warm water and by holding the foot in a dependent position over the edge of the examining table or bed. In our experience the heel or the ear of an infant has not been a satisfactory site for puncture. A Hagedorn needle and a dull blade are both inefficient and unnecessarily painful.

## AN ANTISEPTIC DISPENSER OF LIQUIDS FOR MOISTENING COTTON OR SPONGES\*

GEORGE G. LITTLE, M.E., AND THOMAS B. MAGATH, M.D., ROCHESTER, MINN.

**B**ECAUSE of the numerous requests for a description of the alcohol dispenser for moistening cotton or sponges, which has been in use in the bleeding rooms in the Division of Clinical Pathology at the Mayo Clinic for the past eleven years, this description is submitted.

The apparatus (Figs. 1 and 2) consists of a small, circular basin supported at the upper end of a vertical delivery tube that reaches down to, and connects with, a piston head in the pump cylinder. The tube guides liquid out of the cylinder upward to the basin.

Directly below the basin is a return spring within a telescoping chamber which rests on the top of the rubber stopper. A connecting tube, secured to the lower end of the spring chamber, extends down to the cylinder head to which the cylinder is secured and by which it is supported.

A threaded sleeve secured to the lower end of the spring chamber supports an adjustable stop collar which can be set for a proper distance below the basin to assure delivery of a desired amount of liquid to the cotton or sponge.

In action, when the container is filled with liquid, the return spring holds all movable parts in their normal positions. A sponge is placed in the basin and enough pressure applied to cause the basin, its supporting tube and the piston to be depressed toward the lower end of the cylinder, the tube pushing the piston head down past the filling holes, closing off the liquid contained in the cylinder and forcing the liquid up through the delivery tube to the basin where it comes in contact with the sponge, as required.

A vent hole in the side of the connecting tube, near the upper end of the cylinder, permits the breaking of any vacuum that tends to form in the upper part of the container as the liquid is used.

The complete apparatus is removable for the purpose of refilling the container.

The upper portion of the spring chamber is secured permanently to the lower side of the basin. The delivery tube is threaded into the piston head and hard-soldered to the neck of the basin. The connecting tube is hard-soldered into the lower end of the spring chamber but is threaded into the cylinder head. The knurled adjustable stop is secured in position on its threaded sleeve by a twenty-four pitch thread, with enough friction to keep it in place while in use.

An ordinary large-mouthed bottle serves as a container, with the rubber stopper serving as a support to hold the cylinder out of contact with the bottom of the container.

\*From the Division of Clinical Pathology, Section on Parasitology, The Mayo Clinic.  
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(as estimated by the length of the column) of Hinton indicator is added, taking care to avoid an air bubble between the two liquids. These are now thoroughly mixed by tilting the capillary tube back and forth so that the liquid runs from one end to the other about ten times.<sup>9</sup> Leave a short column of air at each end of the tube, and seal both ends carefully in a flame, preferably a microburner. To the second capillary tube add about five times as much Hinton indicator as serum, mix by tilting and seal as directed above.

Place both tubes in the properly marked test tube and immerse in a 37° C. water-bath for sixteen hours. Centrifuge at high speed for five minutes. Read under the low power of a microscope with the light cut down considerably and the stage inclined at about 30 degrees from the horizontal. The condenser should be lowered about one-half inch from the level of the stage. A strongly positive reaction consists of a firm disc of floccules at the meniscus. Weaker reactions consist of well-defined, dense floccules of various sizes floating at the meniscus. These may sometimes be brought under better observation by gently tapping and then rotating the tube under the lens. In case of doubt, after breaking up the floccules by gentle tapping, recentrifuging causes the aggregates of a positive reaction to re-form at the meniscus. Amorphous and cloudy material at the meniscus does not represent a positive reaction and tends to remain dispersed after recentrifuging. Negative reactions from grossly hemolyzed or contaminated specimens should be reported as "unsatisfactory."

#### RESULTS

During the winter of 1935-36, the American Society of Clinical Pathologists and the U. S. Public Health Service conducted a second evaluation of serodiagnostic tests for syphilis as carried out by state, municipal and private laboratories.<sup>10</sup> Three hundred "unknown" blood specimens were sent out in small lots, approximately two hundred of them from syphilitic patients in various stages of the disease. The rest were from presumably nonsyphilitic individuals, some of them suffering from other diseases. In this series the author's micro-Hinton tests and his capillary Hinton tests gave identical results and received a sensitivity rating of 91.9 per cent and a specificity rating of 100 per cent. The control Hinton series had a sensitivity of 88.8 per cent.\*

From February, 1935 to September, 1936, over 468 micro-Hinton and capillary Hinton tests have been carried out at the Infants' and Children's Hospital in Boston, most of them in a routine way and, with few exceptions (parents and blood donors), on blood obtained by skin puncture from infants and children. The positive tests on the newborn infants of syphilitic mothers were placed in a special category, because they may be influenced by the factor of placental transmission of reacting substances. This group is being made the basis of a separate study. It was possible to check the accuracy of 372 of these tests against the clinical diagnosis and either the regular Hinton, the Wassermann, or, in a few instances, the Kahn test. In infants under ten months of age, the x-rays of the long bones sometimes provided another check.

\*A full report of the results of this evaluation series was published in the January, 1937, issues of the American Journal of Public Health and the U. S. Public Health Service publication, Venereal Disease Information.

## THE OXIDATIVE MICRO-ESTIMATION OF PLASMA TOTAL LIPID\*

ELDON M. BOYD, M.D., KINGSTON, CANADA

IN 1928, Bloor<sup>2</sup> published a method for the microdetermination of total fatty acids and total cholesterol. It was implied in this paper that the sum of the total fatty acids plus the total cholesterol represented the total lipid. Such an assumption has been recently included in certain reference and textbooks, for example that of Beaumont and Dodds.<sup>1</sup> This assumption, however, is not quite correct and *approximates* the truth only in cases where there is very little of phospholipid present in the extracts analyzed.

The method of estimating "total lipid" in this technic is as follows: The lipids are extracted from blood or tissues with alcohol-ether, an aliquot is saponified, then acidified and extracted with petroleum ether. The petroleum ether is evaporated off and the residue of lipids oxidized completely with chromic acid. The number of cubic centimeters of 0.1 N potassium dichromate required for the oxidation is divided by 3.7 and the result indicates the milligrams of "total lipid" in the aliquot analyzed. The factor 3.7 is derived from the fact that ordinarily the cholesterol content of plasma is one-half the total fatty acid, the factor for total fatty acid is 3.6 and for cholesterol 3.92 and hence the factor for "total lipid" was calculated as 3.7. It is a fact that ordinarily there is a fairly constant relationship between plasma total fatty acid and plasma total cholesterol and between the sum of these and the total lipid. This relation is much less constant in tissues.

The factor 3.7 has been found experimentally in the present work to be too high. This may be explained on theoretical grounds. The total lipid of plasma, as we understand it today, is composed of total fatty acid plus total cholesterol plus glyceryl linkages of neutral fat and of phospholipid plus phosphoric acid and nitrogenous groups in the phospholipids. These latter two groups are entirely left out when total lipid is calculated as the sum of the total fatty acid plus total cholesterol.

The factor for converting the titration of the total fatty acid plus total cholesterol in the Bloor technic into total lipid values was determined experimentally in 406 plasma extracts. The total lipid of these extracts was determined by the author's method<sup>3</sup> as the sum of the individual lipids estimated by direct analysis. The estimation of total lipid by this procedure requires the calculation of 10 different lipid values in each extract so that over 4,000 values in all were determined. The estimation of total plasma lipid by the

\*From the Department of Pharmacology, Queen's University.

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Hinton doubtful serum gave a positive micro Hinton reaction by the rapid method. These results correspond very closely with the relative sensitivity of the Hinton and the regular micro Hinton tests incubated for sixteen hours shown in the evaluation series mentioned previously.

#### DISCUSSION

Inasmuch as the author's micro Hinton and his capillary Hinton tests gave identical results, the choice of method should depend entirely on clinical and laboratory convenience. Both tests undoubtedly cannot be performed in the laboratory on a large scale as easily as the regular Hinton test, but the clinician should soon recognize certain advantages which these methods offer. They can be carried out, for example, with the blood donors are being matched for transfusion and with the same serum. The blood in the capillary tube appears to hemolyze less readily than that stored in the larger tubes. For the repeated tests recommended by Dunham<sup>11</sup> and by Faber<sup>1</sup> on infants born of syphilitic mothers, the capillary method is of great value. A study of such infants is being carried out, based on the estimation of the strength of the reactions, a rising titer presumably representing actual infection of the infant, as suggested by Faber in his work with Wassermann titrations.

Since the clinical diagnosis of syphilis particularly in infants and children, is frequently obscure, it is hoped that the development of these methods will, by their convenience to the clinician, encourage the examination of the blood in all cases where the diagnosis is not absolutely certain. In our experience, mothers do not object to having blood taken from their infants for a microtest. In office practice, for example, the specimen of blood can be obtained at the time the finger is pricked for a blood count or hemoglobin determination.

#### SUMMARY

1. Methods for performing micro Hinton flocculation tests for syphilis on blood obtained by skin puncture have been described.

2. In an evaluation series of tests on 300 blood specimens sent out under the auspices of the U. S. Public Health Service, each method demonstrated a sensitivity of 91.9 per cent and a specificity of 100 per cent.

3. In a larger number of tests conducted in the routine of an infants' and children's hospital, the method has been found dependable and practicable.

4. For emergencies, the tests appear to give accurate readings in one hour.

Acknowledgment is hereby made of the valuable assistance of Dr. W. A. Hinton and Miss Genevieve O. Stuart.

#### REFERENCES

1. Kline, B. S. *Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis*, Baltimore, 1932, Williams and Wilkins Company.
2. Chediak, Alexandro. Simplification of Sero Diagnosis of Syphilis by Use of Chediak Micro Reaction From a Drop of Dried Blood, *Arch de med int* 1: 528, 1935.
3. Rein, C. R., and Le Moine, M. Value of Kline Precipitation Test for Syphilis in Applicants for Life Insurance, *Urol & Cutan Rev* 39: 233, 1935.
4. Kahn, R. L. Micro Kahn Reactions, *J. A. M. A.* 87: 2092, 1926.
5. Peterman, M. G. Microprecipitation Test for Syphilis, *Am J Dis C* 1927.

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The method of estimating "total lipid" in this technic is as follows: The lipids are extracted from blood or tissues with alcohol-ether, an aliquot is saponified, then acidified and extracted with petroleum ether. The petroleum ether is evaporated off and the residue of lipids oxidized completely with chromic acid. The number of cubic centimeters of 0.1 N potassium dichromate required for the oxidation is divided by 3.7 and the result indicates the milligrams of "total lipid" in the aliquot analyzed. The factor 3.7 is derived from the fact that ordinarily the cholesterol content of plasma is one-half the total fatty acid, the factor for total fatty acid is 3.6 and for cholesterol 3.92 and hence the factor for "total lipid" was calculated as 3.7. It is a fact that ordinarily there is a fairly constant relationship between plasma total fatty acid and plasma total cholesterol and between the sum of these and the total lipid. This relation is much less constant in tissues.

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The factor for converting the titration of the total fatty acid plus total cholesterol in the Bloor technic into total lipid values was determined experimentally in 406 plasma extracts. The total lipid of these extracts was determined by the author's method<sup>3</sup> as the sum of the individual lipids estimated by direct analysis. The estimation of total lipid by this procedure requires the calculation of 10 different lipid values in each extract so that over 4,000 values in all were determined. The estimation of total plasma lipid by the

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8. Stannous chloride solutions (according to Kuttner and Cohen<sup>2</sup>) Dissolve 10 gm. of stannous chloride in 25 c.c. of concentrated hydrochloric acid. Store in an amber colored, glass stoppered bottle. Dilute 1 c.c. of this stock to 200 c.c. with water. The dilute reagent should be renewed every five days.

### Calcium Determination:

1. Into a conical centrifuge tube of 15 c.c. capacity introduce 1 c.c. of serum and 2 c.c. of 1 per cent ammonium oxalate. Mix thoroughly and let stand for fifteen minutes.

2. Centrifuge at a high speed for five minutes and carefully pour off the supernatant fluid into a test tube to be used subsequently for the determination of inorganic phosphate. Invert the centrifuge tube on a piece of filter paper and let drain for two minutes.

3. To the sediment add 2 c.c. of dilute ammonia water breaking up the mat of calcium oxalate by shaking. Recentrifuge and decant supernatant fluid from sediment. Drain on a piece of filter paper for five minutes.

4. Add 2 c.c. of  $N H_2SO_4$  and break up the mat of calcium oxalate as before. Place in the boiling water bath for two minutes.

5. Titrate the hot solution with standard 0.005  $N$   $KMnO_4$  solution until a faint pink tinge persists. Do not allow the temperature of the solution to fall below  $70^\circ C$  by rewarming in the water bath as often as is necessary. A 20 c.c. buret accurately calibrated to 0.01 c.c. should be used for the titration.

6. For a blank control, titrate 2 c.c. of dilute sulphuric acid warmed as above until a faint pink tinge persists for thirty seconds or longer.

**Calculation.**—(c.c. of 0.005N  $KMnO_4$  used for titration minus c.c. of 0.005N  $KMnO_4$  used for blank) multiplied by 10 = mg. of calcium per 100 c.c. of serum.

### Inorganic Phosphate Determination:

1. To 2 c.c. of the residual diluted serum from the calcium determination add 12 c.c. of a 2.3 per cent trichloroacetic acid solution. Mix and filter through an ashless (P free) filter paper.

2. Pipette 7 c.c. of the clear filtrate (equivalent to  $\frac{1}{2}$  c.c. serum) into a 6 by 1 inch test tube. Into a similar tube pipette 7 c.c. of working phosphorus standard. To both add 2 c.c. of molybdic sulphuric reagent solution and immediately thereafter 1 c.c. of dilute stannous chloride solution.

3. Mix and compare in the colorimeter after sixty seconds.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 5 = \text{mg. inorganic phosphorus per 100 c.c. of serum.}$$

Set unknown at 25 mm. and divide reading of standard by five.

This procedure has been used by the author for the past year. A series of 65 comparative analyses were conducted on serums ranging in calcium content from 7.1 to 16.6 mg. per cent and in phosphorus content from 2.68 to 6.62 mg. per cent. The maximum variations between the above method and the results obtained by the Clark-Collip method were plus 0.3 mg. and minus 0.3 mg. with an average difference of plus 0.032 mg. The maximum variations between the above method and results obtained by the Kuttner-Cohen method were plus 0.10 mg. and minus 0.06 mg. with an average difference of plus 0.02 mg.

### REFERENCES

1. Clark, B. P., and Collip, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.
2. Kuttner, T., and Cohen, H. R. Micro Colorimetric Studies, *J. Biol. Chem.* 75: 517, 1927.

Pipette 0.2 c.c. of serum, using the pipette with a capillary tip, and place 0.1 c.c. of serum in each of two small test tubes. The pipettes should be rinsed three times with physiologic salt solution between serums, and dry sterilized at the end of the day. Relative cleanliness and freedom from bacterial contamination of the pipettes and test tubes is important. Inactivate the serum at 55° to 56° C. for thirty minutes. Add to one tube 0.1 c.c. of regular Hinton indicator and to the other 0.5 c.c. Should less than 0.1 c.c. of serum be available for the second tube, a 1:5 ratio of serum to indicator may be maintained with smaller quantities. Mix by tapping in an inclined position and incubate at 37° C. in the water-bath for sixteen hours.

After incubation, place the tubes in the properly numbered centrifuge tubes and centrifuge at high speed for five minutes. Read directly or in the concave mirror of a microscope. A *positive* reaction consists of clearing of the liquid, with *definite* floccules at the meniscus in *either* tube. Barely visible granules and amorphous, stringy material are of no significance. Strongly positive reactions may cause the floccules to collect into a firm disc at the meniscus. In case of doubtful reactions, shake the tube moderately and re-centrifuge. Positive floccules will then re-form and nonspecific aggregates due to bacterial or other contamination tend to disperse. A small proportion of specimens give a positive reaction only in the second tube. A *negative* reaction consists in the absence of clearing of the liquid and absence of floccules at the meniscus in *both* tubes. A *doubtful* reaction consists in a questionable increase in the size of the granules originally present in the "indicator."

*B. The Author's Capillary Hinton Test (0.05 c.c. of serum).*—To collect the blood sample, a capillary glass tube is used instead of a Wright's tube. This capillary tube measures 11.5 cm., or slightly less, in length and about 1.25 to 1.5 mm. in inside diameter.\* After filling the tube with blood from a finger or great toe to within about 2 cm. of the opposite end, this empty end is sealed in a flame or with a generous cap of sealing wax, labeled with an adhesive sticker, and placed in a protective test tube.

In the laboratory, the capillary tube is centrifuged toward the sealed end at high speed for five minutes. With the serum separated from the clot, the tube is broken just above the level of the blood cells, and the serum is run into another capillary tube. A little practice with a graduated pipette will give the operator's eye skill in approximating the 0.05 c.c. of serum which is recommended for the capillary test. (The exact amount is not important.) This tube is sealed at one end with melted sealing wax or in a flame, placed with the sealed end downward in a properly numbered test tube filled with water at 56° C., and the serum is inactivated in a water-bath at 56° C. for thirty minutes.† The capillary tube is removed, the seal broken, and a small amount of serum (approximately 0.01 c.c.) is run into a second capillary tube. To the first tube, containing the larger amount of serum, an equal quantity

\*Purchased from Friedrich and Dimmock, Millville, N. J. The capillary tubes are cleaned in a manner similar to that used for the Wright's tubes described above. Recently, somewhat shorter and wider glass tubes fitted with rubber caps have been employed successfully for the collection of the blood samples and for carrying out the tests.

†Having separated the clot from the serum by centrifuging the blood sample, serum and clot may be inactivated together, thus saving one step.

minutes the  $\Delta D$  was 0.20 at  $\lambda$  6600  $\mu$ . It is thus evident that the optimum blue green color of the Liebermann-Burchard reaction is unstable. The point of maximum optical density of this reaction cannot be determined quantitatively by means of simple color comparison.\*

The measurement of the evanescent color due to cholesterol is further complicated by the fact that the extracts of blood are often brown or purple, especially when they have dried too long or at too high a temperature. These extraneous colors are often deep and exhibit maximum optical densities at wave lengths which alter the color of the solution and yield indeterminate errors in optical color comparison.

A filter transmitting light between  $\lambda$  6400  $\mu$  and 6600  $\mu$  excludes these colors. The advent of the Evelyn colorimeter† permits the application of the foregoing facts to a simplified technique for the determination of cholesterol in small quantities of whole blood. The extraneous colors can be accurately excluded; a color standard is unnecessary; the transient color of the Liebermann-Burchard reaction can be measured at the time of its maximum intensity

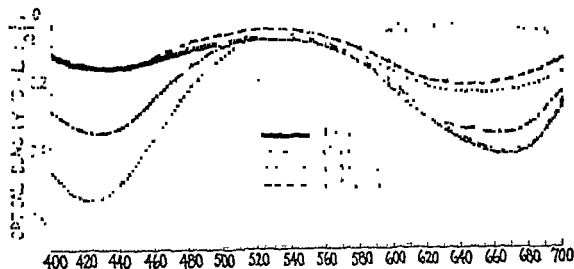


Chart 1

#### METHOD

##### Reagents and apparatus:

1. Ethyl alcohol—95%
2. Ether, anhydrous
3. Chloroform, anhydrous
4. Acetic anhydride, C.P.
5. Concentrated sulphuric acid, C.P.
6. Fohn micropipettes, 0.1 c.c.
7. Evelyn colorimeter and tubes
8. Centrifuge tubes

#### PROCEDURE

Mix 6 c.c. of 95 per cent ethyl alcohol and 2 c.c. of anhydrous ether in a clean, dry centrifuge tube. Add 0.1 c.c.† of whole blood, drop wise from a Fohn micropipette. Cork tightly and shake vigorously for one minute. Let the tube lie semihorizontally with an even distribution of the sediment for thirty minutes (Sackett<sup>16</sup>). Centrifuge for three minutes and decant the supernatant fluid into a standard Evelyn tube. Evaporate to dryness in an

\*Confirmed by personal communication from the Color Measurements Laboratory, Massachusetts Institute of Technology.

†0.2 c.c. can be used.

TABLE I

RESULTS OF MICRO-HINTON AND CAPILLARY HINTON TESTS COMPARED WITH THE CLINICAL DIAGNOSES AND THE RESULTS OF OTHER TESTS

	NEGATIVE	POSITIVE	DOUBTFUL	TOTAL
Microtest and capillary test	302	62	8	372
Clinical agreement	301	57	3 <sup>a</sup>	361
Clinical disagreement	1 <sup>b</sup>	5 <sup>c</sup>	5 <sup>d</sup>	11
Agreement with other tests <sup>e</sup>	134	57	2	193
Disagreement with other tests <sup>e</sup>	1 <sup>c</sup>	4 <sup>c</sup>	2 <sup>f</sup>	7

a, Under treatment for syphilis.

b, Patient six days of age. Micro-Hinton positive at seven weeks. Congenital syphilis.

c, Pneumonia, scarlet fever, lung abscess, phlyctenular conjunctivitis, and ratbite fever. The last gave a positive Hinton reaction as well.

d, Two from patients with deferred diagnoses, three from nonsyphilitic patients.

e, Micro Hinton proved correct.

f, Hinton negative.

g, Wassermann, Kahn, regular Hinton, or, in infants, x-rays of long bones.

The reactions agreed with the clinical picture in all but eleven cases, five of them apparently being false positive reactions, five doubtful, and one false negative. The last occurred in an infant aged six days,<sup>\*</sup> that at seven weeks of age developed a positive micro-Hinton test and clinical evidence of syphilis. One of the five false positives came from a case of ratbite fever (a spirochetal disease), the Hinton test also being positive. The other four were slight reactions, misinterpreted as positive, and came from febrile children. There were eight doubtful reactions, three from children who had been under treatment for congenital syphilis, and three from nonsyphilitic patients. The diagnosis in two was not determined. Subsequently, four of the five patients who had false positive microreactions were reexamined and gave negative microtests.

For purposes of comparison with the regular Hinton test, capillary Hinton tests were performed on eight serums giving doubtful Hinton reactions on blood from adults whose clinical diagnoses were later investigated. Five gave definitely positive capillary Hinton tests, one was doubtful and two were negative. The doubtful reactor had had a chancre in 1931, and a negative Hinton test for three years. One negative reactor had been under treatment and observation since 1922 and had had nine negative Wassermann tests. All the positive reactions corresponded with definitely syphilitic patients.

Nineteen micro-Hinton and capillary Hinton tests were performed on serums giving Hinton tests which were hard to read. Five positives, 2 doubtfuls, and 9 negatives agreed with the Hinton test. In 3 others there was relative agreement. These were negative micro-Hinton tests from clinically nonsyphilitic patients, the Hinton test being doubtful.

Since the objection has been raised that the Hinton test requires too long an incubation period for use in emergencies such as blood transfusions, micro-Hinton and capillary Hinton tests were performed in which the serums were inactivated twenty minutes, incubated with the Hinton indicator for thirty minutes, and centrifuged ten minutes. In other words, the tests were carried out from beginning to end in a little over one hour. In a series of 92 Hinton positive and 29 Hinton negative serums, the tests agreed in all but one case, where the microtest was positive and the regular Hinton test negative. One

\*Roentgenograms of the long bones negative at the same time.

original method of Bloor and the method of Schoenheimer and Sperry, with the exception that the Evelyn colorimeter was used in the latter to considerable advantage

A test of accuracy would be a comparison with the Windaus macrogravimetric method the error of which is generally believed to be within the limits of  $\pm 2$  per cent<sup>14</sup> Since the digitonin method of Schoenheimer and Sperry varies in most instances only  $\pm 1$  to  $\pm 5$  from that of Windaus, we compared our results directly with those of the former

Furthermore, recovery within  $\pm 3$  per cent of added amounts of cholesterol to blood of known cholesterol content was obtained This was done on repeated samples

The effect of temperature on the color formed by the action of acetic anhydride and  $H_2SO_4$  in cholesterol is important At room temperature the original green color changes to a yellow brown In general, our results tend to show that the higher the temperature the earlier the color reaches a maximum, and the earlier it fades Variation of the amount of  $H_2SO_4$  used, likewise, affects the speed of color development, at room temperature the greater the amount of  $H_2SO_4$  used, the earlier the development of the blue green color

This is entirely in accord with the findings of Schoenheimer and Sperry As a consequence of the effect of temperature on color, we have found repeatedly that a maximum color can be obtained in the cold (refrigerator) in forty five minutes This procedure obviates the effect of natural temperature changes which might take place within the laboratory

# CONCLUSION

A simple, rapid, quantitative method for the determination of cholesterol in 0.1 cc of blood is presented

# REFERENCES

- 1 Grigaut, A. Procédé Colorimétrique Le Dosage de la Cholestérine dans l'organisme, *Mémoires de la Soc de Biol* 68 791, 1910
- 2 Liebermann, C. Ueber das Oxycholesterin, *Ber d deutsch chem Gesellsch* 18 1803, 1883
- 3 Burchard, Hans. Beiträge zur Kenntnis des Cholesterins. Diss Rostock 1889 Reviewed in *Chem Zentralbl* 61 25, 1906
- 4 Autenrieth, W, and Funk, A. Ueber kolorimetrische Bestimmungsmethoden Die Bestimmung des Gesamtcholesterins im Blute und in Organen, München med Wchnschr 60 1243, 1913
- 5 Henes, E, Jr. Cholesterinemia, *Proc N Y Path Soc* 13 100, 1913 1914
- 6 Myers, V C, and Gorham, F D. Chemical Composition of the Blood in Health and Disease IV Cholesterol, *Post Grad M J* 29 938, 1914
- 7 Csonka, F A. A Critique of Certain Data on the Content of Cholesterol and Fatty Substance in the Blood, Together With a Modification of the Colorimetric Method for Estimating Cholesterol, *J Biol Chem* 24 431, 1916
- 8 Gettler, A O, and Baker, W. Chemical and Physical Analysis of Blood in Thirty Normal Cases, *J Biol Chem* 25 211, 1916
- 9 Bernhard, A. The Determination of Cholesterol, *J Biol Chem* 35 10, 1918
- 10 Bloor, W R. Determination of Small Amounts of Lipid in Blood Plasma *J Biol Chem* 77 23, 1923
- 11 Roehrig, Armin. Verbesserter Apparat zur Milchfett Bestimmung nach Gottlieb Roese, *Ztschr f Untersuch d. Lebensmitt* 9 231, 1905

6. Sellek Azzi, A., and del Frade, A.: Modification of Meinicke Clarification Reaction (M.K.II) Using Small Amounts of Serum; Value in Infancy, Arch. de med. inf. 5: 6, 1936.
7. Cumming, H. S., and others: The Evaluation of Serodiagnostic Tests for Syphilis in the United States: Report of Results, J. A. M. A. 104: 2083, 1935; also Ven. Dis. Inform. 16: 189, 1935.
8. Hinton, W. A.: Hinton Test for Syphilis, Third Modification, J. LAB. & CLIN. MED. 18: 198, 1932.
9. Ponsold, A.: Micro-Method for the Quantitative Analysis of Small Amounts of Serum, Deutsche Ztschr. f. d. ges. gerichtl. Med. 23: 46, 1934.
10. The Evaluation of Tests for Syphilis in State and Local Laboratories, J. A. M. A. 105: 286, 1935.
11. Dunham, E. C.: The Diagnosis of Congenital Syphilis in the New-Born, Am. J. Dis. Child. 43: 317, 1932.
12. Faber, H. K., and Black, W. C.: Quantitative Wassermann Tests in Diagnosis of Congenital Syphilis: Clinical Importance of Fildes' Law, Am. J. Dis. Child. 51: 1257, 1936.

## A COMBINED MICRODETERMINATION OF CALCIUM AND PHOSPHORUS IN BLOOD SERUM\*

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THE determination of blood serum calcium is usually of clinical significance only in conjunction with the simultaneous evaluation of serum phosphate. By methods at present in use in clinical laboratories, from 3 to 5 c.c. of serum are required for these two tests. The following procedure enables the combined determination of calcium and phosphate in 1 c.c. of serum. In principle, it involves precipitating blood serum calcium as calcium oxalate (as in the Clark-Collip modification of the Kramer-Tisdall method<sup>1</sup>) and titrating the latter with standard permanganate solution in the presence of acid. The residual diluted serum from the calcium determination is deproteinized with a dilute trichloroacetic acid solution and inorganic phosphate is determined in the filtrate thereof by a modified Kuttner-Cohen procedure.<sup>2</sup>

*Reagent Solutions Required.*—(All chemicals of C.P. grade.)

1. 1 per cent ammonium oxalate solution.
2. Dilute ammonia water. Dilute 20 c.c. of 28 per cent aqua ammonia to one liter with distilled water.
3. Sulphuric acid, approximately normal. Add 28 c.c. of concentrated sulphuric acid (66° Bé) to 970 c.c. of distilled water.
4. Standard 0.005 N potassium permanganate solution. Store in an amber colored, glass-stoppered bottle. Restandardize every week. 1 c.c. equivalent to 0.1 mg. calcium.
5. Molybdic-sulphuric reagent. Dissolve 37.5 gm. of P-free sodium molybdate in a solution of 140 c.c. of concentrated sulphuric acid in 800 c.c. of water. Dilute to 1 liter and mix.
6. 2.3 per cent trichloroacetic acid. Dissolve 23 gm. of trichloroacetic acid in 200 c.c. of water and dilute to 1 liter.
7. Phosphorus standards. Dissolve 1.0450 gm. of pure, dry  $\text{KH}_2\text{PO}_4$  in water to make one liter of solution. Add 1 c.c. of chloroform to preserve. 1 c.c. contains 0.2382 mg. of phosphorus. To make the working phosphorus standard, dilute 10 c.c. of this stock to 1 liter; 1 c.c. of the working standard contains 0.00238 mg. of phosphorus.

\*From the Department of Laboratories, Israel-Zion Hospital.  
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estimation of minute quantities of iodine. A small quantity of starch solution containing potassium iodide is placed in the bulb and a vacuum is created by

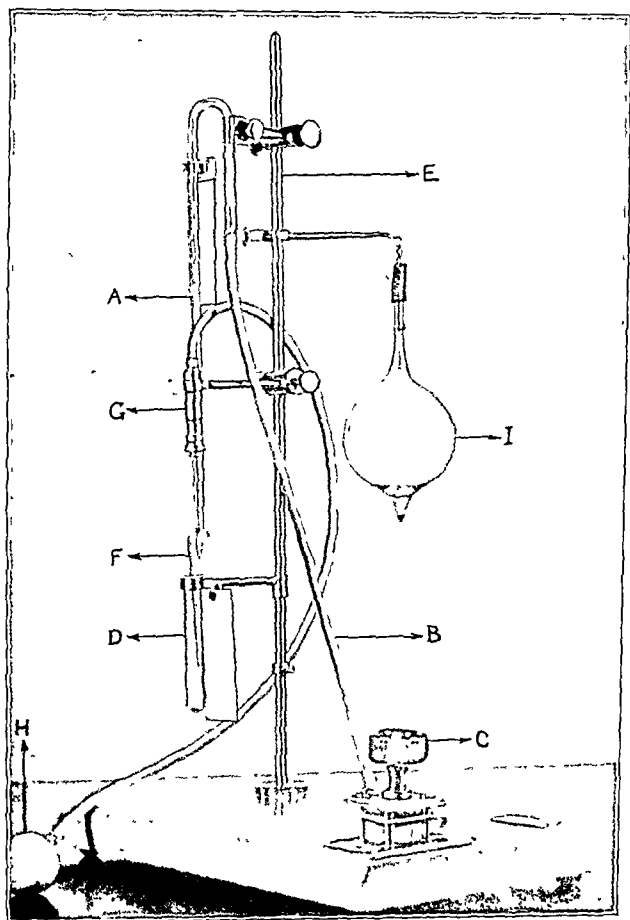


Fig 1

driving out the air with heat. The opening in the bulb is sealed by means of a short piece of rubber tubing containing a glass bead. The exit or entry of air is permitted by sliding the rubber tubing with the thumb and finger slightly

## A MICROMETHOD FOR THE DETERMINATION OF BLOOD CHOLESTEROL\*

MICHEL PIJOAN, M.D., AND CARL W. WALTER, M.D., BOSTON, MASS.

THE interpretation of blood cholesterol values has been uncertain, in part, because of the technical difficulties encountered in routine clinical analysis. Grigaut<sup>1</sup> first developed the colorimetric method based upon the Liebermann-Burchard<sup>2, 3</sup> reaction. Autenrieth and Funk,<sup>4</sup> Henes,<sup>5</sup> Myers and Gorham,<sup>6</sup> Csonka,<sup>7</sup> Gettler and Baker,<sup>8</sup> Bernhard,<sup>9</sup> and Bloor<sup>10</sup> have modified the extraction method of cholesterol from blood in an attempt to produce color reactions suitable for colorimetry. The use of ether and alcohol as a solvent for the extraction of cholesterol, originally proposed by Roesse and Gottlieb,<sup>11</sup> and subsequently applied by Bloor<sup>12</sup> to blood, has been established by Fowweather<sup>13</sup> as preferable to dry extraction. Despite the use of meticulous technic in this method, routine determinations are hampered by the development of extraneous brown, purple or yellow colors which modify the characteristic green color, rendering visual colorimetry difficult or inaccurate. In 1934, Schoenheimer and Sperry<sup>14</sup> demonstrated that the chromogenic substances present in the fractions of extracts in small amounts of blood, could be excluded if cholesterol were isolated as a digitonide. This communication presents an accurate, rapid and relatively simple procedure for the determination of the total cholesterol in small amounts of blood without using digitonin precipitation. Only 0.1 c.c. of serum or whole blood is required, and it is possible, therefore, to utilize fingertip blood for the analysis.

### GENERAL CONSIDERATIONS

The green color of the Liebermann-Burchard reaction is transient, attaining its maximum intensity after a variable interval; is photosensitive; and has its maximum optical density at  $\lambda$  6600  $\mu$ . Simultaneous reactions due to the lipoids which are present yield colors which would affect the adsorption curve of the blue green color at other wave lengths. It is, therefore, of importance to observe the intensity of the blue green color at its maximum optical density at the wave length of 6600  $\mu$ .

The "fading" of the blue green color, which has been repeatedly observed, introduces the question of the optimum time for the determination of the color present. In Chart 1 the adsorption curves are drawn to represent their change with time at room temperature (25° C.).

Curve 1 illustrates a  $\Delta D$  ( $D = \log 10 \frac{1}{t}$ ) of 0.42 at 6600  $\mu$ ; four minutes later Curve 2 was obtained in which the  $\Delta D$  was 0.36. After twenty minutes the color had faded so that the  $\Delta D$  was 0.22, and after twenty-five

\*From the Surgical Laboratory of the Peter Bent Brigham Hospital.  
Received for publication, September 23, 1936.

## SUMMARY

1 A chemical test for pregnancy was performed on urine according to the technique of Visscher and Bowman

2 Cases were used with Friedman controls and in addition the urine of known pregnant women was tested

## CONCLUSIONS

The Visscher Bowman test for pregnancy is of questionable clinical value

I wish to express my appreciation to Miss Laura Amatucci and Mr Alfredo Alfano for their kind cooperation in the performance of this test

## REFERENCES

- 1 Visscher, J Paul, and Bowman, Donald E Proc Soc Exper Biol & Med 31 460, 1934
- 2 Mencken, J G Deutsche med Wchnschr 60 1837, 1934
- 3 Schenck and Tran Jewish Hospital of Brooklyn Personal Communication

## A FURTHER IMPROVED CONGO RED TEST FOR AMYLOIDOSIS\*

ALBERT TARAN, B A, STATEN ISLAND, N Y

SINCE Bennhold<sup>1</sup> described the Congo red test, an ever increasing number of clinicians have resorted to it in the attempt to determine the presence of amyloidosis. The progress made in this field has not been marked since as yet no accurate laboratory method has been devised to ensure reliable results in determining the percentage of absorption of the dye.

The method as presented by Bennhold proceeds as follows: ten cubic centimeters of a 1 per cent solution of Congo red are injected intravenously in the postabsorptive state. Four minutes, and then one hour, later about 10 c c of blood are drawn and allowed to clot. The tubes are centrifuged and the serum removed and placed in cups of a colorimeter, whereby readings are made, using the four-minute specimen as a standard. He found that absorptions of 60 per cent or over were obtained in cases of amyloidosis and that values up to 60 per cent were obtained in cases of nephrosis.

This test has proved of great value in determining the presence and extent of amyloidosis. However, it has fallen short in that it has not yielded accurate results in cases where the slightest trace of turbidity or hemolysis was present in one or both of the tubes.

Friedman and Auerbach<sup>2</sup> solved this problem in part by adding ethyl alcohol to precipitate the proteins and extract the red dye. Thus any hemoglobin which may have been present was precipitated along with the other serum proteins, leaving a clear solution which could be read in the colorimeter.

\*From the Department of Pathology, Sea View Hospital, Oscar Auerbach, M.D., Pathologist.

incubator at 37° C., inclining the tube at an angle of 15° and using a current of warm, dry air to hasten the process. Add 10 c.c. of anhydrous chloroform, 4 c.c. of acetic anhydride, and 0.1 c.c. of concentrated sulphuric acid. Place the tube in an electric refrigerator (Temp. -5° C. to +5° C.). At the end of forty-five minutes remove the tubes and read, using a filter (Corning Glass) transmitting light from  $\lambda$  6300  $\mu$  and  $\lambda$  6600  $\mu$ . Several readings should be taken in order to ascertain if the color has developed to its maximum intensity. The results can be charted as a calibration curve or as a table (Table I).

TABLE I

BLOOD CHOLESTEROL VALUES FOR GALVANOMETER DEVIATIONS FROM BLANK READING. A SOLUTION OF ACETIC ANHYDRIDE AND CHLOROFORM IS USED AS A BLANK, AND THE GALVANOMETER ADJUSTED TO 100

GALVANOMETER DEVIATION	CHOLESTEROL MG. PER CENT	GALVANOMETER DEVIATION	CHOLESTEROL MG. PER CENT
48	400	67	232
49	390	68	222
50	380	69	214
51	372	70	206
52	362	71	200
53	352	72	192
54	342	73	184
55	334	74	178
56	328	75	172
57	318	76	164
58	308	77	158
59	300	78	152
60	292	79	144
61	282	80	138
62	274	81	132
63	266	82	124
64	258	83	118
65	250	84	112
66	240	85	106
		86	100

TABLE II

DETERMINATION OF TOTAL CHOLESTEROL IN SERUM BY SCHOENHEIMER AND SPERRY, BLOOR, AND THE METHOD PRESENTED

SERUM NO.	SCHOENHEIMER AND SPERRY MG. PER 100 C.C.	BLOOR METHOD		NEW METHOD	
		AMOUNT MG. PER 100 C.C.	DEVIATION FROM SCHOENHEIMER AND SPERRY	AMOUNT MG. PER 100 C.C.	DEVIATION FROM SCHOENHEIMER AND SPERRY
1	160	182	+22	164	+4
2	200	210	+10	202	+2
3	182	208	+26	186	+4
4	174	192	+18	180	+6
5	176	199	+13	178	+2
6	162	184	+22	160	-2
7	200	210	+10	198	-2
8	204	209	+5	202	-2
9	146	150	+4	150	+4
10	130	140	+10	128	-2
11	192	194	+2	190	-2
12	120	132	+12	122	+2
13	98	104	+6	97	-1
14	260	280	+20	262	+2
15	210	219	+9	212	+2

## DISCUSSION

In developing this method several different modifications of technic have been tested with several hundred known and unknown samples using the

TABLE II

BEFORE ACETONE ADDITION PER CENT ABSORPTION	AFTER ACETONE ADDITION PER CENT ABSORPTION	PER CENT DIFFERENCE
25	27	+2
42	45	+3
60	57	-3
35	35	0
85	81	-4
80	82	+2
90	87	-3
65	61	-4
75	79	+4

tion In cases of high absorptions, such as 80 per cent or 90 per cent, the one hour specimen was so light in color after dilution that an accurate comparison could not be made Table III gives a comparison between these two methods

TABLE III

ALCOHOL METHOD PER CENT ABSORPTION	ACETONE METHOD PER CENT ABSORPTION	PER CENT DIFFERENCE
20	22	+ 2
90	70	-20
75	55	-20
45	38	-7
72	60	-12
90	65	-25
85	60	-25
58	45	-13
85	55	-30
80	52	-28

The best means of proof of the reliability of the acetone method is in the correlation between laboratory findings and clinical and postmortem examinations The acetone method, on the whole, has been found to check more closely with clinical and postmortem findings Much work is being done in this hospital at present in correlating laboratory and postmortem examinations to determine more exactly the percentage of absorption which could be used as a reliable diagnostic aid in amyloidosis

## SUMMARY

- 1 An improved Congo red test for amyloidosis is presented
- 2 More accurate results are obtained, according to preliminary unpublished data This method checks more closely with clinical and postmortem findings
- 3 A small dilution of the serum is necessary
- 4 Comparisons are made between the original method and the new acetone method, as well as the alcohol and acetone methods

## REFERENCES

- 1 Bennhold H Über die ausscheidung Intravenös Einverleibten Kongorotes, Deut-sches Arch f klin Med 142 32, 1923
- 2 Friedman, M M and Auerbach Oscar An Improved Congo Red Test for Amyloidosis J LAB & CLIN MED 21 93 1935
- 3 Ernst, Z, and Forster, J Über Die Bestimmung des Blutbilirubins, Klin. Wchnschr 3 2386, 1924
- 4 Dragstedt, Carl A, and Mills, Moore A The Employment of Oxalated Plasma in the Bromsulphalein Dye Retention Test, J LAB & CLIN MED 21 1306, 1936

12. Bloor, W. R.: The Determination of Cholesterol in Blood Serum, *J. Biol. Chem.* 24: 227, 1916.
- Bloor, W. R., Pelkan, K. F., and Allen, D. M.: Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma, *J. Biol. Chem.* 102: 191, 1922.
13. Fowweather, F. S.: The Determination of the Amount and the Composition of the Fat of Faeces. I. Investigation of a "Wet" Method and Comparison With the "Dry" Method, *Brit. J. Exper. Path.* 7: 7, 1926-27.
14. Schoenheimer, R., and Sperry, W. M.: Micromethod for Determination of Free and Combined Cholesterol, *J. Biol. Chem.* 106: 745, 1934.
15. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, *J. Biol. Chem.* 115: 60, 1936.
16. Sackett, G. E.: Modification of Bloor's Method for Determination of Cholesterol in Whole Blood or Blood Serum, *J. Biol. Chem.* 64: 203, 1925.

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## AN APPARATUS FOR MICROTITRATIONS\*

ESPECIALLY ADAPTED FOR THE ESTIMATION OF BLOOD IODINE

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ANALYTICAL procedures involving microchemical principles such as those employed in the estimation of the iodine content of the blood, require equipment of extraordinary delicacy and precision. In an attempt to fulfill this requirement, the titration apparatus described in this paper and illustrated by Fig. 1 was devised.

The buret (*A*) is constructed from a 0.2 c.c. graduated Kahn pipette, bent in the form of a U and with the tip drawn out to a fine capillary bore. The rubber tube (*B*) connects the inverted end of the buret with a rubber bulb of 10 c.c. capacity, compression or expansion of which is controlled by the screw clamp (*C*). This contrivance permits of precise regulation of the reagent within the buret. The tube (*D*) which receives the titrating fluid is held by a movable support which can be retained at a desired level by the serrations on the upright metal rod (*E*).

During the use of the apparatus, the titrations are facilitated by the fine glass mixing rod (*F*) which is suspended from the loose-fitting plunger of the metal air-compression cylinder (*G*). When a titration is in progress, the delivery of the liquid from the buret is controlled by means of the screw (*C*) and the admixture of the fluid in the tube (*D*) with that which drops from the tip of the buret is obtained by the rapid up-and-down movement of the rod (*F*) accomplished by alternate compression and relaxation of the rubber bulb (*H*) held between the thumb and fingers. The plunger within the cylinder (*G*) is drawn upward by the suction of the bulb and drops by gravity. The movable white glass plate attached to the buret is helpful in making the buret readings, and a similar plate behind the tube (*D*) aids in the recognition of the end-points.

The glass bulb (*I*) is an accessory which is employed for the detection of iodine in the air, a form of contamination which might be a cause of error in the

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\*From the Department of Pathological Chemistry, University of Western Ontario.  
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In every doubtful case roentgen examinations should be made, particularly of the gastrointestinal tract. Gallbladder visualization is of little or no use.

In surgical jaundice, early operation is important.

In medical jaundice, protection of liver parenchyma by a suitable diet (and dextrose injections when needed) is the essential thing.

**FUNGI, Direct Microscopical Examination of the Skin** Swartz J H, and Conant, N F  
Arch Dermat & Syph 33 291 1936

The treatment of scrapings from the skin with 5 per cent potassium hydroxide, followed by washing with water and staining in lactophenol and cotton blue, makes possible the easy determination of the presence of fungi. This method of preparing microscopic specimens for examination is simple in that it adds only two steps to the more common method using potassium hydroxide and is effective in that the fungi are definitely stained while the various confusing artefacts are eliminated from the picture.

This is particularly true of the mosaic growth which occurs commonly in preparations made with potassium hydroxide. This material does not seem to be the result of treatment with potassium hydroxide since it can be found in scales treated with xylene or with chloral hydrate and acacia. It was found to be soluble in ether, 95 per cent alcohol, absolute alcohol and phenol. It could not be stained with sudan III or scarlet red or blackened with osmic acid. While fungi were readily stained with lactophenol and cotton blue, mosaic material which occurred in the same preparation did not stain and was, in fact, entirely eliminated. In the presence of polarized light there was no evidence that the mosaic material was composed of crystalloid forms.

Whatever the nature of the mosaic growth the authors feel that they have presented sufficient proof that it is not a fungus. The staining of normal fungi in scales with lactophenol and cotton blue, the failure to find partially degenerated forms in the same preparation in which both mosaic material and fungi occurred and the failure to find morphologic connections between normal hyphae and these mosaic forms seem to disprove convincingly the theory that this material is a degenerate form of fungus.

The method follows

Lactic acid	1 c c
Phenol crystals	1 gm
Glycerin	2 c c
Water (distilled)	1 c c

To this may be added 0.5 per cent cotton blue (C4B Poirrier), which was considered by Langeron to be the best type.

When scales were gently heated in a drop of this liquid on a slide and a cover glass was pressed on them, it was seen that fungi, when present, stained more deeply than did the epidermal cells. The varied thicknesses of the scales, however, hampered the effectiveness of the clearing action of the lactophenol, and it was found advantageous to subject the scales to preliminary clearing in a 5 per cent solution of potassium hydroxide. After this preliminary treatment the scales were transferred to a watch crystal and washed with water. When the action of the potassium hydroxide was stopped after two or three minutes of washing the scales were gently heated in a drop of the lactophenol cotton blue mixture, and a cover glass was pressed on the preparation. They were then mounted in clear lactophenol, and the preparation was pressed out under a cover glass. The fungi were heavily stained, showing the protoplasmic content and hyaline outer sheath.

To obtain permanent preparations of the scales stained with lactophenol and cotton blue, all that is necessary is to wipe off the excess medium around the cover glass and cement the cover to the slide with Noyer's cement. Another method of obtaining permanent preparations was found to be that of mounting the stained scale in chloral hydrate and acacia.

Distilled water	50 c c
Chloral hydrate	50 gm
Glycerin	20 c c
Acacia	30 gm.

to one side away from the glass bead. In order to test for the presence of iodine in the atmosphere, a sample of the suspected air is allowed to enter the bulb by this maneuver, and by shaking, the air and reagent are brought into contact with one another. Thus the presence of iodine, even in very dilute concentration, will cause the reagent to turn blue.

The authors have found the apparatus described above to be useful for the estimation of the iodine content of the blood and urine in connection with an iodine tolerance test described elsewhere.<sup>1</sup>

#### REFERENCE

1. Watson, E. M., and Barber, A. S.: An Iodine Tolerance Test for the Investigation of Thyroid Function, *Endocrinology* 20: 358, 1936.

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### EXPERIENCES WITH THE VISSCHER-BOWMAN TEST FOR PREGNANCY\*

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IN 1934 Visscher and Bowman<sup>1</sup> reported a method for the determination of pregnancy by directly testing the urine with a number of chemical substances. The test was performed by taking 1 c.c. of urine and to it adding five drops of 5 per cent methyl cyanide, five drops of 1 per cent hydrogen peroxide, five drops of 1 per cent phenylhydrazine hydrochloride, and five drops of concentrated hydrochloric acid and placing in a boiling water-bath for twenty-five minutes. If a darkening plus the formation of a precipitate occurred, the test was considered positive.

Thereafter, Mencken<sup>2</sup> reported a small number of cases in which he had tried this test and although somewhat enthusiastic, the poorly controlled experiment apparently yielded nothing definite. We attempted to carry out this technique on a series of cases with the Friedman test as a control and also ran a series of tests on known pregnant women who entered the Maternity Wards of the Hospital. Although every precaution was taken to carry out this procedure exactly as defined by Visscher and Bowman, including the fact that reducing substances might even interfere, we feel that we must discard this test as being of questionable clinical value in the determination of pregnancy.

Our statistics which led us to such conclusions are as follows: In nineteen cases with Friedman controls the test agreed in 57.8 per cent of the cases and disagreed in 42 per cent of the cases. On fifty-six known pregnant women at term, the test was positive in 64.3 per cent, negative in 36 per cent, and doubtful in 0.5 per cent. Results similar to our own were also obtained by Schenck and Tran.<sup>3</sup>

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\*From the Laboratories of Metropolitan Hospital. (Andrea Saccone, M.D., Director.)  
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2 Origin in regard to the type of time (nevoid or inflammatory) and of cells (physiologic, or adult, pathologic, or neoplastic)

3 Anaplasia, involving change in (a) physiology, cell differentiation or dyskeratosis (type, extent, relation) or (b) morphology (size, shape, staining characteristics and nuclear changes)

#### 4 Broder's classification

5 Associated changes in the cutis (a) active inflammatory protective zone (character, cytology, vascular changes), (b) sequelae of changes in the cutis degeneration (fibrosis, etc.)

For diagnosis, one should note the cell type, with its character of development and manner of infiltration, the dyskeratosis, with its extent and type, and the character of the inflammatory protective zone.

### GRANULOPENIA, Further Studies in Experimental, Parker F P, and Kracke, R R Am J Clin Path 6 41 1936

Since experimental agranulocytosis can be consistently produced in animals with benzene and some of its oxidation product, a consideration of a possible mechanism is discussed.

A resume of the accelerating action of glutathione on the rate of cell division suggests the possibility of this substance influencing bone marrow activity, and further suggests that its depletion would lead to bone marrow aplasia with resulting leucopenic states.

Experiments are presented in which agranulocytosis was produced in rabbits by the daily injection of benzene, with the leucopenic animals showing a marked depletion of the biologically active or reduced glutathione in bone marrow and blood.

The glutathione changes in the blood of twenty patients with various hematologic disorders showed a marked increase in those cases characterized by pathologic leucocytic stimulation, a marked decrease in those showing bone marrow aplasia, and with no change in those showing physiologic leucocytosis.

It is suggested that the reduced form of glutathione plays an important role in regulating normal bone marrow activity, and that depletion of this substance in the blood stream or bone marrow may lead to various leucopenic states.

Determinations carried out on various preparations of liver extract show the presence of a large amount of reduced glutathione, suggesting that this substance may be partially responsible for the therapeutic effect of liver extract in some of the leucopenic diseases.

### URINE, New Method for Determination of Sugar in, Gugliucci A. Rit Med Naples 42 389, 1936

The method following depends upon the reduction of potassium ferri-cyanide to potassium ferrocyanide by dextrose.

#### Reagent—

Potassium ferri-cyanide	9.90 gm
Sodium carbonate, anhydrous	60.85 gm
Distilled water to make	1,000 cc

The reagent is stable.

#### Method—

1 Place 10 cc of reagent in a large test tube and heat to boiling.

2 Add urine drop by drop, heating after each addition, until the yellow color disappears.

#### Calculation—

$$\frac{10}{\text{cc of urine used}} = \text{grams of dextrose per liter}$$

Their method consisted of diluting the serum with alcohol in the proportion of 1:5. This dilution we have now found to be too great to yield very accurate results, especially in cases where only a small amount of dye was present in the serum.

The present method devised in this laboratory has been found to be both more practical as well as more accurate in determining the percentage of absorption of the dye. Equal parts of serum and acetone are used. Acetone precipitates the proteins, extracts the dye, yields a clear solution and does not affect the intensity of the original color to any great extent. Similar use of acetone in precipitating blood proteins was made by Ernst and Förster<sup>3</sup> in determining the icteric index on serum, and by Dragstedt and Mills<sup>4</sup> in the bromsulphalein test for liver function.

*Method.*—Ten cubic centimeters of a 1 per cent Congo red solution are injected intravenously in the postabsorptive state. Four minutes, and then one hour, after the injection of the dye, about 10 c.c. of blood are drawn and placed in clean, dry test tubes. It is now best to wait until clotting and retraction take place. Centrifuge the tubes and remove the serum. Take 4 c.c. of serum from each specimen and add 4 c.c. of acetone. Shake the tubes well and centrifuge them at a moderate speed for about ten minutes. Removal of the proteins may also be accomplished by filtering. The clear solutions are placed in microcups of a colorimeter and compared. The four-minute specimen, acting as a standard, is placed at 20 mm., while readings are made with the one-hour specimen.

*Calculation:*

$$100 - \left( \frac{\text{Reading of four-minute spec.}}{\text{Reading of one-hour spec.}} \times 100 \right) = \text{per cent absorption.}$$

Table I shows the importance of removing hemoglobin from the serum. In some cases one-hour specimens were obtained which showed more intense redness than the four-minute specimen, due to a great degree of hemolysis.

TABLE I

DIRECT READING OF SERUM PER CENT ABSORPTION	AFTER ACETONE PRECIPITATION PER CENT ABSORPTION	PER CENT DIFFERENCE
55	30	-25
50	40	-10
20	45	+25
20	35	+15
25	45	+20
5	90	+85
10	70	+60
30	65	+35

In order to check upon the validity of the acetone method, we have taken unhemolyzed serum and added Congo red to it in various concentrations. These were read both before and after acetone addition. Table II gives the results of these tests and shows the close correlation obtained.

Before adopting the acetone in place of the alcohol method, we conducted a series of tests to which both methods were applied. The alcohol method was found to be reliable only when there was a small percentage of absorption, and when there was sufficient dye in the serum which could be read after great dilu-

**THROMBOCYTOPENIA**, Congenital, Sanford, H. N., Leslie, E. I., and Crane, M. M. *Am J Dis Child* 51 1114, 1936

A case of congenital thrombocytopenia is reported in which both mother and infant, besides showing marked thrombocytopenia, showed an increased disintegrative ability of the platelets that kept the mechanism of coagulation in balance.

**LEAD POISONING**, Concentration of Lead in the Urine in the Diagnosis of, Shuels, D. O. *Med J Australia* 1 559, 1936

The concentration of lead in the urine of 29 subjects of compensatable lead poisoning has been determined, and also of 55 subjects who were fit and exposed to generally similar hazards.

The average value in the case of fit subjects was 0.12 milligram per liter.

The average value in the case of subjects suffering from compensatable lead poisoning from whom samples were taken while they were still exposed to the hazard or within a few days of leaving it, and who had undergone no treatment previous to reporting such, was 0.23 mg per liter.

Out of 114 patients, 24 showed concentrations equal to or greater than 0.20 mg per liter, of these, 17, or 70.8 per cent, were suffering from compensatable lead poisoning.

Thirteen subjects showed concentrations equal to or greater than 0.25 mg per liter, of these, 12, or 92.2 per cent, were suffering from compensatable lead poisoning. Of the subjects with compensatable lead poisoning, 50 per cent showed urinary lead concentrations equal to or greater than 0.25 mg per liter.

The results confirm those of Kehoe, Thamman and Cholak, who considered that lead poisoning could be expected when the urinary lead excretion was 0.21 mg per liter, and of Brown, who found that 50 per cent of the subjects of lead poisoning had urinary lead concentrations above 0.20 mg per liter.

**LYMPHOBLASTOMAS** The Lymphatoid Disease, Krumphaar, E. B. *J. A. M. A.* 106 286, 1936

Although the group of diseases under consideration includes several of different or unknown etiology, and a pathologic histology that is recognizably different in most, the great similarity or overlapping of many of the clinical pictures makes it convenient and advisable to include them in a group designation.

For this heterogeneous group, a noncommittal term "lymphomatoid diseases" is suggested instead of the scientifically inaccurate and progress inhibiting designation of "lymphoblastoma" with its unjustifiable indication of neoplasm. It is further suggested that the latter term be reserved for the use for which it was coined, "a tumor derived from the lymphoblast," and not distorted out of sense to supply a clinical need.

In spite of, or rather in view of, the overlapping and often baffling clinical pictures met the classification and the individual diagnoses as far as possible should be on a pathologic basis. It is more desirable to leave the diagnosis of cases in which this is impossible as tentative or unmade than to make unwarranted groupings under a single head for the sake of giving a label to a greater number of individual cases.

An analysis of 150 cases of these lymphomatoid and related diseases in the autopsy records of the University and Philadelphia General Hospitals has brought out various items of etiologic and pathologic interest. Useful light on the essential nature of the diseases however, has not been forthcoming.

Phagocytosis of tumor cells may occasionally be so marked as to require consideration as a factor in tumor resistance.

The lymphomatoid diseases are practically all alike in having a fatal prognosis though the duration may extend from a few days to many years. With few exceptions they are peculiarly susceptible to and improved by radiation treatment. The relative resistance of the reticulosos to radiation may prove useful in segregating this group.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**LYMPHOCYTE, A Previously Undescribed Granule Within the, Gall, E. A.** Am. J. M. Sc. 191: 380, 1936.

Description is given of the presence of a motile, refractile globule which is normally present in a fairly constant percentage of the lymphocytes of peripheral blood observed in wet preparations. The bodies vary in size from a point to about 0.5 micron in diameter and appear in approximately 34 per cent of the circulating lymphocytes. They occur for the most part in normal lymphocytes but have been noted in pathologic cells as well. The numbers of granule-bearing lymphocytes remain quite constant in the same individual from day to day. They do not appear spontaneously or disappear in the standing blood spread. One or two are observed in a cell, rarely more. The spherule is either mitochondrial nor one of the neutral red staining bodies previously described as existing in lymphocytes. It is probably neither of a lipid nature or a portion of the centrosphere. It appears to be a hitherto undescribed constituent of the cytoplasm of the lymphocyte.

The spherules are markedly refractile, appear solid black at one focus and have a colorless glassy sheen at another in the unstained state. Ordinarily a single granule and much less often 2 or 3 are present in one lymphocyte. In several cases of chronic myelogenous and lymphatic leukemia and one undiagnosed splenomegaly with anemia in an infant there have been observed as many as 8 in one cell, but never more. Only 1 or 2 cells among 200 to 400 lymphocytes counted in each of these patients displayed this number. The characteristics of the globular particles were exactly the same as those in the ordinary lymphocyte. They were all approximately the same size (0.3 micron). When two granules were present in a single cell, as occurred in about 30 to 40 per cent of the spherule containing lymphocytes, one was more often much smaller than the other.

These intracellular inclusions are actively motile. In preparations examined promptly after withdrawal, their motility is most striking. They course about usually within the perinuclear zone, often under or over the nucleus, in a zigzag fashion. Occasionally they obtrude toward the periphery of the cell but generally they remain quite close to the nucleus. As they approach the nuclear Hof they frequently impinge upon other less motile cytoplasmic structures (mitochondria and segregation apparatus) lending to these added impetus and receiving in return retardation and deflection of their momentum and course. As has been noted, the motility of the other visible cytoplasmic elements was neither as rapid nor as extensive as that of the refractile bodies.

**JAUNDICE, Painless, Ottenberg, R.** J. A. M. A. 104: 1681, 1935.

The important diagnosis is between medical and surgical jaundice.

There is no sure method of distinguishing obstruction from suppression of bile (liver cell injury).

Determining whether the van den Bergh reaction is direct or indirect does not help.

The icterus index is preferred to the quantitative van den Bergh test for following the curve of bilirubinemia.

Extremely high blood bilirubin most commonly occurs in hepatic degeneration.

High percentages of blood cholesterol and cholesterol esters point to obstruction, but on rare occasions they may occur in hepatic degeneration.

Low percentage of cholesterol esters points to hepatic degeneration. But a normal or even an elevated percentage does not rule out degeneration.

A positive galactose tolerance test indicates hepatic degeneration. A normal test does not exclude degeneration.

In jaundice, tyrosin in the urine points to liver degeneration or malignancy. 'Large amounts point to acute liver autolysis. Its absence has little significance.



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**TRICHOMONAS VAGINALIS**, Contrast Stain for, Miller, J. R. J. A. M. A. 106: 616, 1936.

A drop of 0.1 per cent safranin used as a diluent for the pus to be examined furnishes a useful contrast.

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**HYPERTENSION**, Benign and Malignant, and Nephrosclerosis, Kimmelstiel, P., and Wilson, C. Am. J. Path. 12: 45, 1936.

A. Benign hypertension and benign nephrosclerosis may show a parallel development but in the early stages are not casually related. In the later stages, however, there may be a reciprocal relationship, i.e.:

1. Hypertension acts as an accelerating factor on the development of arteriosclerosis.

2. Arterial and arteriolar sclerosis of the kidney, when severe enough to produce impairment of renal function, may give rise to "renal" fixation of the hypertension. Such cases are termed "decompensated benign nephrosclerosis," since clinical and histologic evidence shows that the impairment of function is of true renal origin.

B. Malignant hypertension and malignant nephrosclerosis, on the other hand, show a definite correlation.

1. On clinical and histologic grounds malignant hypertension is to be regarded as a primary generalized vascular disease of which malignant nephrosclerosis represents the "renal end-stage." Cases are described in which death occurs from malignant hypertension before the renal end-stage is reached.

2. When malignant hypertension progresses to the stage of malignant nephrosclerosis, the condition is clinically and histologically characteristic, as described by Volhard and Fahr. The main objection to their classification is the existence of so-called "borderline" cases, which are neither clinically nor histologically characteristic. Of these cases, in our interpretation, one group consists of cases of malignant hypertension in which death occurs before the renal phase develops; the other group comprises older subjects in whom the malignant hypertension is less fulminant and may be superimposed on benign nephrosclerosis.

3. Endarteritis in its diffuse form is regarded as the most characteristic histologic sign of malignant hypertension. Arteriolitis (arteriolar necrosis) is more closely related to the terminal renal failure than to the hypertension itself.

4. Various hypersensitive states may act as precursors of malignant hypertension. Evidence is presented that diffuse glomerulonephritis may similarly be associated with or followed by malignant hypertension, thereby explaining the occurrence of the "specific" vascular lesions in the kidney in diffuse glomerulonephritis.

5. A study of the relation of periarteritis nodosa to malignant nephrosclerosis provides suggestive evidence that two factors are necessary for the development of malignant hypertension, namely, a preexisting hyperactivity or "sensitivity" of the arteries, on which is superimposed a precipitating factor, allergic or otherwise.

**EPITHELIOMA OF SKIN**, Histologic Evidence of, Satenstein, D. L. Arch. Dermat. & Syph. 33: 48, 1936.

A guide for study of microscopic sections is as follows:

1. Relation of the epithelial proliferation (a) to the epidermis (developing above or below the surrounding epidermis; developing within or beyond the basal cell layer); (b) to the cutis infiltrating into the superficial or deep cutis; infiltrating en masse or disseminated or diffuse.

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**PURPURA, Thrombocytopenic, The Value of the Prognostic Venom Reaction in,** Peck, S. M., Rosenthal, N., and Erf, L. A. *J. A. M. A.* 106: 1783, 1936.

An intradermal moccasin snake venom test has been used as a prognostic measure in essential thrombocytopenic purpura hemorrhagica.

Persistence of a positive reaction to successive tests, or a reversal to a negative reaction, is of value in determining the trend of the purpuric state.

Subcutaneous injections of moccasin snake venom have been employed as a therapeutic measure in chronic purpura hemorrhagica. It apparently has been of value in twenty-two of the thirty-four cases in which it has been used.

The effect of subcutaneous venom injections and the trend of the intracutaneous venom test are important for the indication and prognosis of splenectomy.

The intradermal skin test known as the prognostic venom reaction consists of the injection intradermally of 0.1 c.c. of 1:3,000 standardized moccasin venom, with a control of 0.1 c.c. of physiologic solution of sodium chloride. The test is read in one hour. A positive reaction is manifested by capillary rupture with diffusion of blood into the tissues. The absence of capillary rupture constitutes a negative reaction. Diffusion of blood at the site of injection after twelve hours is regarded as a delayed positive reaction. A positive reaction indicates the presence of a purpuric state and a close association with a thrombocytopenia. Clinical improvement is shown by a change from a positive to a negative reaction.

**ICTERUS GRAVIS, Certain Types of,** Ross, S. G., and Waugh, T. R. *Am. J. Dis. Child.* 51: 1059, 1936.

The facts brought out by this study lead to the view that icterus gravis may be divided into two main groups: (a) the hemolytic and (b) the obstructive type. In many cases the jaundice results from a combination of the two factors. The first type is the more common. The cause of the hemolysis is unknown. The course of the disease is rapid. The jaundice appears early. As illustrated previously, obstructive jaundice may supervene and persist after the hemolysis has ceased. In other cases the obstructive jaundice may disappear and the hemolytic jaundice may persist. When the obstructive jaundice is marked, a hemorrhagic diathesis may appear. The authors have brought forward evidence against the use of the term erythroblastosis of the newborn for the hemolytic type of jaundice, as the appearance in the blood and tissues of the excess of embryonic red cells does not appear to be a primary factor in the causation of the disease but merely a secondary temporary response to the severe anemia. The prognosis in this type of the disease must be guarded on account of the severe anemia, the tendency to collapse and the risk of hemorrhage from the obstructive jaundice. Should the patient recover from the acute disease, the danger of cerebral lesions, resulting in spasticity, cerebral diplegia and mental deficiency, appears to be well founded.

The purely obstructive type of icterus gravis seems to be a rarer condition than the type just discussed. The jaundice is less intense, it usually appears later, the course of the disease is more favorable and anemia does not develop.

The authors' observations on the hemolytic type of icterus gravis support the view of Parsons that some factor in the blood of the mother or infant favors excessive hemolysis and that the embryonic cells appearing in the blood are the result and not the cause of the resulting anemia. This lays open to doubt the wisdom of accepting the term erythroblastosis of the newborn, as used by de Lange, Blackfan, Clifford and others, for this condition.

The treatment of the two types is obviously different. For that reason, an early diagnosis, which is only possible by a careful taking of the history, physical examination and, most important of all, a complete examination of the blood, is essential. In the hemolytic type, the severe anemia must be treated by early and often frequent transfusions. The authors have had no experience in the use of injections of blood serum. In the treatment of the obstructive type of jaundice with no accompanying anemia, transfusions are obviously not indicated, and the treatment is principally dietary.

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### Clinical Implications of Modern Physiologic Hematology

UNTIL recently, hematology was almost entirely morphologic since little was known concerning the physiology of blood cell formation, circulation, and destruction. Such physiologic facts as were proved were seldom correlated with morphologic findings gained from examining the circulating blood. The clinical management of blood dyscrasias has changed completely within the past few years, due largely to advances in physiologic knowledge. Our idea concerning the embryology of the blood cells, the factors necessary for the growth and nutrition of cells, the state of the bone marrow, and the metabolism of hemoglobin have changed entirely due largely to the research in this country of Sabin, Doan, Whipple, Minot and Murphy, Castle, and many other workers. In no field of medicine has fundamental research work been more rapidly adapted to clinical problems to the great benefit of the patient.

In the Beaumont Lectures for 1936, Dr. Charles S. Doan, who has had an active part in the basic research in hematology during the past fifteen years, reviews the important work done by him and his associates and applies his experimental findings to blood problems in man.

Much valuable research work has been lost to the patient for the lack of clinical interest and interpretation on the part of the research worker. Dr. Doan fortunately combines the research and clinical ability and interest necessary to apply original findings to clinical problems. This monograph illustrates especially the great value of the clinical application of fundamental research. The two lectures concern (1) Blood cell origin and maturation, and (2) blood cell distribution and destruction. Dr. Doan's contribution will be read with great interest and profit by everyone concerned with modern physiologic hematology.

### Guide-Book in Practical Obstetrics†

WHILE, as the title indicates, this book is intended to serve as a guidebook for the author's students, it might well be included as an essential part of the obstetrical equipment of any practitioner engaged in this field of medicine, and particularly should it be within easy reach of those who practice obstetrics only occasionally.

Many ponderous tomes have been written on this subject, but few of them contain more practical and useful advice. Concise, yet not laconic, evidencing on every page an extensive and well digested experience, this book is truly what it sets out to be—a guidebook. And those who follow it will not go far wrong, no matter what the situation with which they are confronted, for within its less than two hundred pages it comprises a wealth of practical obstetrics.

The book is divided into three sections. Part I presents obstetrical technique as practiced by the author and taught to his students, and includes the management of complications.

Part II presents in detail the Fay Arnold conception of eclampsia and the treatment used by these authors with such conspicuous success.

Part III consists of "Obstetrical Review Clinics"—a presentation of illustrative cases which comprise, as it were, a condensed postgraduate course.

No one will make a mistake in the purchase of this book.

The reviewer has but one criticism. There is so much of real and practical value in this small volume that it is regrettable that it is not equipped with an index.

\*Clinical Implications of Modern Physiologic  
Professor of Medicine and Director of the Department of  
of Medicine, Ohio State University, Columbus, Ohio  
Cloth, 160 pages, 12 charts, 12 photomicrographs  
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Charles S. Doan, Pro-  
fessor, College  
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†Guide-Book in Practical Obstetrics By J. O. Arnold, M.D. Professor of Obstetrics  
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\*J. A. M. A. 108: 1054, March 27, 1937.

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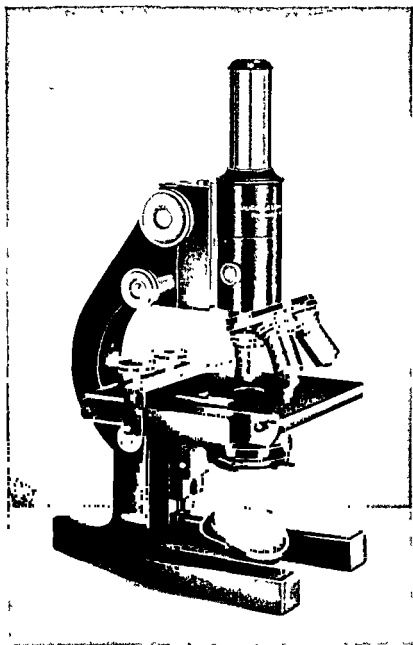
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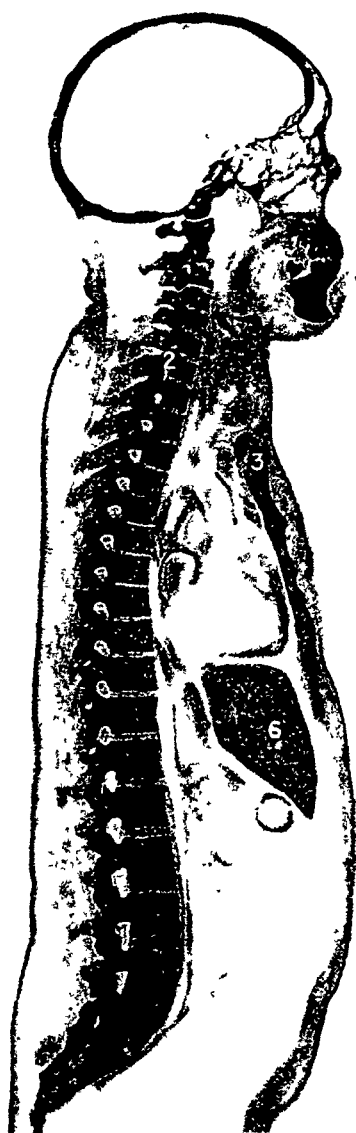
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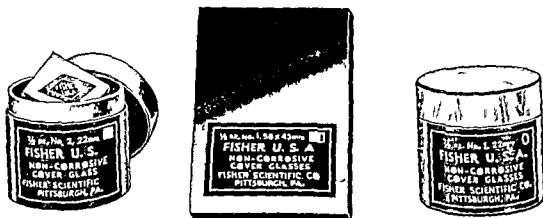
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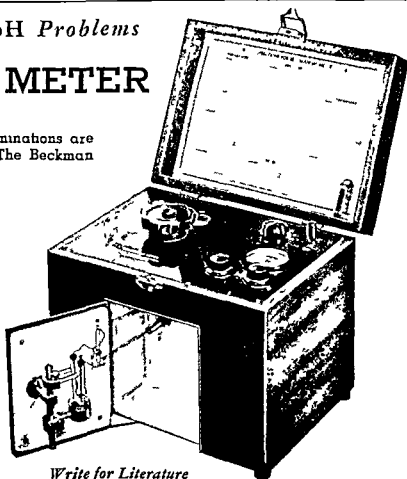
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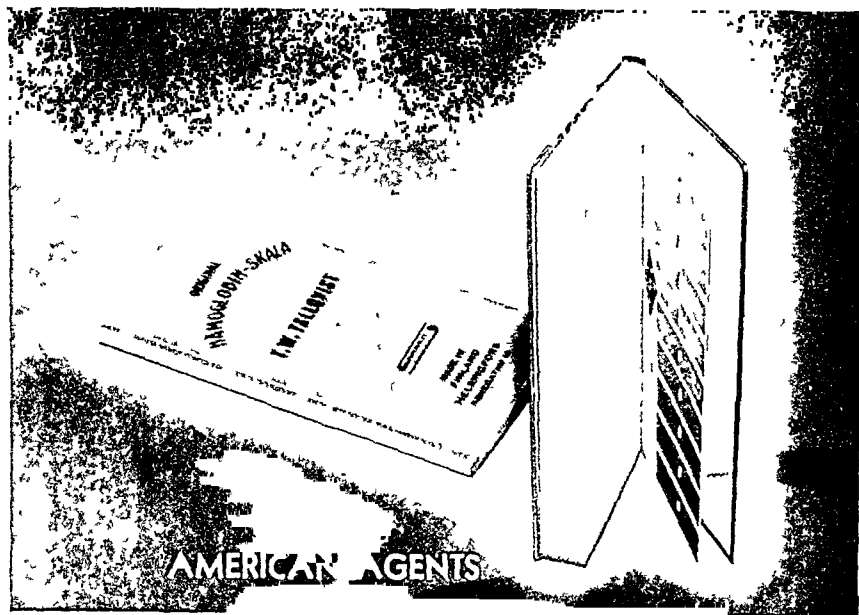
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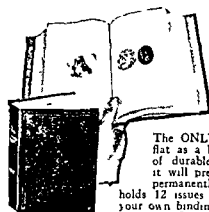
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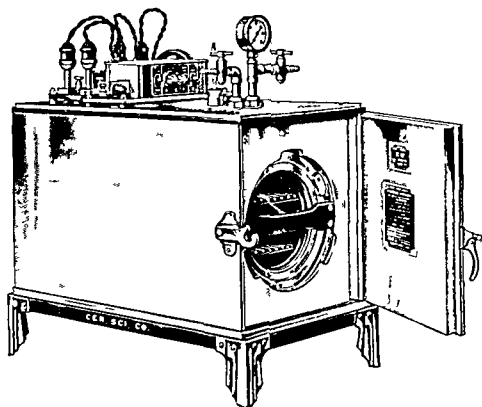
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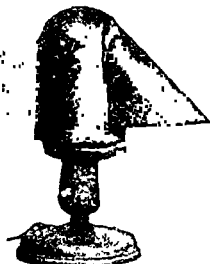
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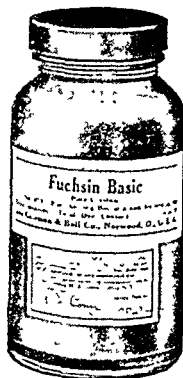
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# *The Journal of Laboratory and Clinical Medicine*

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VOL. 22

AUGUST, 1937

No 11

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## *CLINICAL AND EXPERIMENTAL*

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### EXPERIMENTAL STREPTOCOCCUS INFECTIONS IN RABBITS FOR THERAPEUTIC INVESTIGATIONS\*

---

JOHN A. KOLMLER, M.D., AND ANNA M. RULE, PHILADELPHIA, PA.

---

THE work described in this paper is the result of an effort to produce experimental streptococcus infections in rabbits suitable for therapeutic investigations. The problem has been the production of a primary local lesion permitting direct observation as well as the production of a focal lesion associated with bacteriemia and metastatic infections in order to approximate the usual severe streptococcus septicemia of human beings. In other words, the production of bacteriemia alone does not appear to be sufficient, as the problem of chemotherapy is not only disinfection of the blood but much more importantly, disinfection of the primary and secondary infections of the fixed tissues.

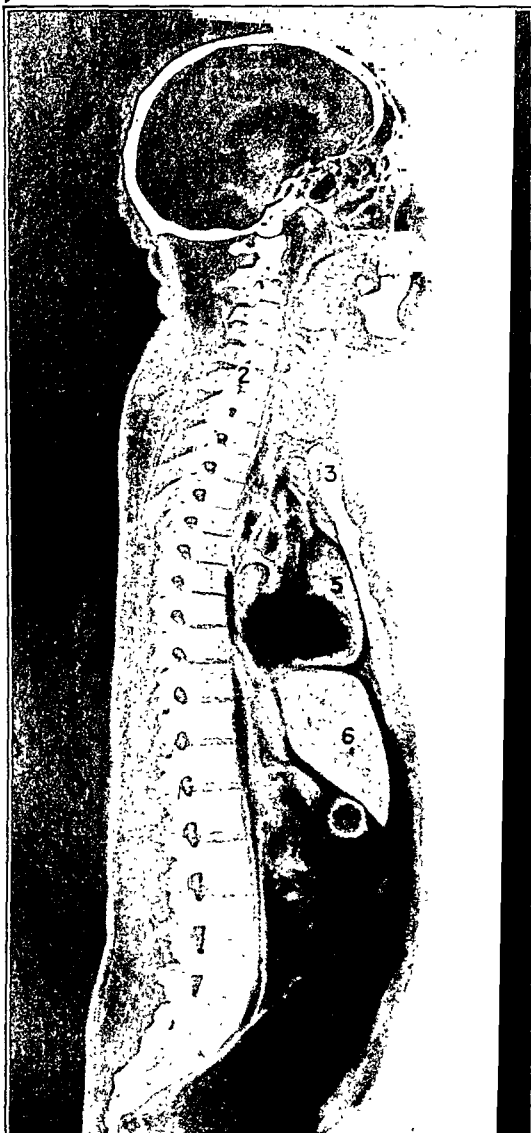
Mice inoculated intraperitoneally with highly virulent strains of streptococcus have been usually employed, but in our experience either a too rapidly fatal septicemia or no septicemia at all has usually resulted. Morgenroth<sup>1</sup> has employed mice inoculated subcutaneously for the production of a focal lesion and septicemia which has proved a better procedure than intraperitoneal infections<sup>2</sup> but in our experience a sufficiently virulent strain produces fatal infection too rapidly for therapeutic investigations since the severity of the infection does not usually permit the administration of repeated doses of the agent under study and may mask any slight curative activity insufficient to produce complete disinfection in one or two doses at daily intervals.

Since we have found Goodner's<sup>3</sup> intradermal infection of rabbits with pneumococcus<sup>4</sup> a very helpful experimental lesion for serum and chemothera-

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\*From the Research Institute of Cutaneous Medicine.  
Received for publication, December 16, 1936.

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*Observations*—Beginning two to four hours later, frequent observations of the local intradermal lesion are made, particularly as to its size, color, amount and consistency of swelling along with morning and evening temperatures and daily leucocyte counts. In the combined intradermal and systemic infections, the joints are examined for signs of suppurative arthritis among animals surviving seven to ten days or longer when these lesions are usually detectable by clinical examination.

*Blood and Lesion Cultures*—Using a small syringe and needle about 0.2 to 0.3 c.c. of blood was removed aseptically from a marginal ear vein and planted in a tube of hormone broth. No attempt was made to plate blood for numerical counts. These cultures were made daily or at frequent intervals.

The local lesion cultures were made daily by aspirating fluid aseptically with a small needle and inoculating tubes of hormone broth. Since the lesions usually contained but little fluid (usually much less than observed in pneumococcus lesions) these were not always successful and particularly after the fourth or fifth day. Joints were aspirated and cultured in the same manner, using needles sufficiently large for the aspiration of pus.

#### THE LOCAL LESION PRODUCED BY INTRADERMAL INFECTION

The lesion usually makes its first appearance about 2 to 4 hours later as an inflamed swollen area from 1 to 2 cm. in diameter. It progresses rapidly, reaching its maximum size in twenty-four hours and measuring about 5 by 6 cm. At this time it is markedly swollen by the accumulation of cloudy serous exudate and leucocytes and of red, angry appearance sometimes hemorrhagic and especially in the central portions, fading to pinkish color at the periphery (Fig. 1). The administration of certain new chemical agents sometimes produced a temporary exacerbation of these changes.

The lesion usually reached its maximum size in twenty-four to thirty-six hours after infection, when regression usually begins. The lesion does not leak serum, shows no tendency to break down and but rarely becomes contaminated with staphylococci or other organisms from the skin.

In from four to six days most of the swelling has disappeared but a local abscess with pus and superficial crusting frequently developed at this time. Healing usually occurred in from ten to fourteen days after inoculation.

For purposes of record the severity of the local lesion was conveniently recorded as + + + +, to express the maximum of severity, with + + +, + +, and + expressing progressing less severe lesions, and - designating practically complete healing.

In no instance was this local infection found to produce arthritis or metastatic infection in the other organs and tissues.

None of our animals succumbed from this local infection alone.

In no instance were the lymphatic glands of the groin or axillae demonstrably enlarged or suppurative.

*Microscopic Examination of the Local Lesion*—Histologic examination of tissues removed twenty-four hours after inoculation, when the lesions were at

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WARREN T. VAUGHAN, M.D., Editor  
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About twelve hours after infection the temperature rises to about  $103^{\circ}$  or  $104^{\circ}$  F., reaching the maximum ( $103^{\circ}$  to  $105^{\circ}$  F.) at the end of twenty-four to thirty-six hours followed by rapid regression to pre-infection levels at the end of forty-eight to seventy-two hours as shown in Chart 1 representative of the usual temperature curve.

Occasionally in severe infections this drop in temperature has been followed by secondary elevations, each one becoming progressively less as shown in Chart 2, although not always accompanied by demonstrable exacerbation of the local lesion.



Fig. 2.—A photograph of a focal lesion forty-eight hours after intradermal infection

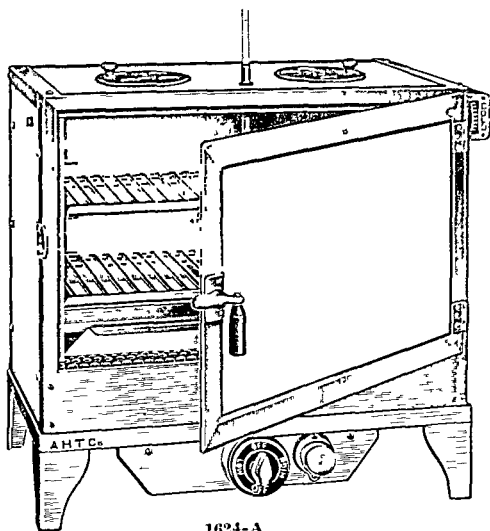
*Leucocyte Counts.*—As is well known the leucocyte counts on rabbits are generally regarded as subject to great fluctuation. The average count on our normal fasting animals has varied from about 7,000 to 12,000 per c.mm., but repeated counts on individual rabbits undergoing handling has shown that it is difficult to establish a relative normal for any individual animal.

Daily counts before and after inoculation however, have shown leucocytosis usually reaching a maximum in from twenty-four to forty-eight hours after infection and following fairly closely the temperature elevations. As shown in Chart 1, the leucocyte counts generally reach from 14,000 to 16,000 per c.mm. at this time, falling to normal levels by the third or fourth day but subject to secondary increase in the case of those animals having secondary elevations of temperature (Chart 2).

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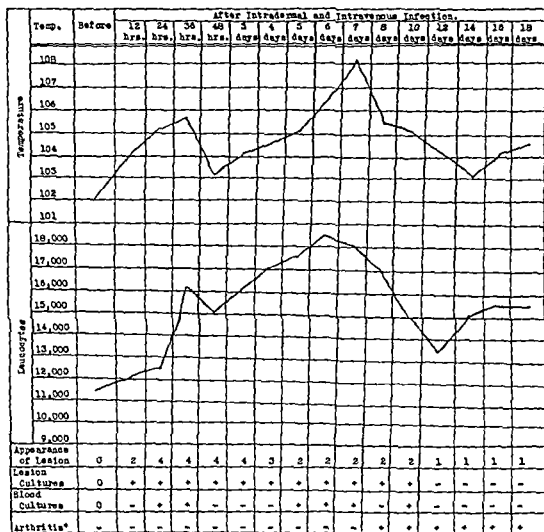
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## THE COMBINED INTRADERMAL AND SYSTEMIC INFECTION

As previously stated this was produced by the intradermal injection of 0.6 c.c. of eighteen-hour hormone broth cultures in divided amounts followed four hours later by the intravenous injection of 0.5 c.c. of the same culture.

The local lesions and cultures were closely similar to those described. In other words, the supplementing intravenous inoculation did not appear to render the local lesions any less severe but positive blood cultures were much more frequent and especially during the first three to four days following infection.



\* Left hind knee, ankle and hip joints (cultures positive).

Chart 3.

The temperature and leucocytic changes however, were more irregular and usually much more pronounced as shown in Chart 3 representative of the series.

This combined infection however, was usually much more severe than the intradermal infection alone since many of the animals succumbed within twenty-one days and sometimes as early as forty-eight hours after infection.

The most characteristic features were the almost invariable development of bacteremia in twenty-four hours, persisting for three to six days or longer, followed by the development of suppurative arthritis among the majority of surviving animals in from five to ten days after infection and persisting for

peutic investigations, we have inquired into the possibilities of using a similar method with streptococcus. With sufficiently virulent strains of hemolytic types, severe focal lesions have been produced but rarely associated with bacteriemia and, consequently, not fatal in outcome. As in the case of the pneumococcus infection, the local lesions however, permit direct observations on the influence of any therapeutic agent being administered. By combining intradermal with intravenous injections of virulent streptococci, a severe local and systemic infection may be produced usually resulting in bacteriemia and suppurative arthritis and also constituting a satisfactory experimental infection for therapeutic investigations.

#### EXPERIMENTAL METHODS

*Streptococcus Cultures.*—The hemolytic streptococcus (Group A) used in these experiments was originally isolated from a throat three years ago. Since then additional strains of sufficient virulence have been obtained from various human sources, and recently from a case of puerperal sepsis. Only strains sufficiently virulent for mice to produce rapidly fatal septicemia following the intraperitoneal injection of 0.001 c.c. or less of eighteen-hour hormone broth cultures have been found satisfactory. Virulence has been maintained by frequent mouse or rabbit transfers. It is apparent, however, that the natural resistance of the adult rabbit to intradermal inoculation is much higher than that of the mouse to intraperitoneal infection. For example, while 0.001 c.c. of eighteen-hour hormone broth culture may produce rapidly fatal septicemia in the latter corresponding to about 0.05 c.c. per kilogram, well-pronounced intradermal lesions required the injection of 0.6 c.c. in rabbits weighing from 1,800 to 2,400 gm. Furthermore, the resistance of adult rabbits to intradermal infection alone, as well as to combined intradermal and intravenous infection, has been found to vary, as some animals developed more severe infections than others when infected with approximately similar amounts of culture per body weight, but the variations in therapeutic experiments have been readily controlled by including a sufficient number of untreated control animals.

*Rabbits.*—Full-grown healthy rabbits (1,800 to 2,400 gm.) have been found more satisfactory than younger animals. White or light gray animals are preferred. The hair of the abdominal skin was removed with barium sulphide. After preliminary temperature and leucocyte observations the streptococci were injected.

*Production of Intradermal Infection.*—The site of infection was the midline about midway between the thorax and pelvis; 0.6 c.c. of eighteen-hour hormone broth culture is injected intradermally being divided into three injections of 0.2 c.c. closely spaced since the skin is too thin to accommodate more than this amount of inoculum. As shown microscopically however, it is impossible to inject 0.2 c.c. strictly intradermally because the layer of epidermis is too thin to accommodate this amount of inoculum. As a result, the larger portion is deposited beneath the epithelial layer.

*Production of Intradermal and Systemic Infection.*—The intradermal infection is given in the same manner as above, followed four hours later by a single intravenous injection of 0.5 c.c. of the same culture.

ferred that this particular serum contained therapeutic amounts of antibody for the particular strain of streptococcus employed

So far no experiments have been conducted in the treatment of rabbits infected by the combined method of intradermal and intravenous infection. It remains to be determined, therefore, whether or not antistreptococcus sera have any appreciable effect upon the bacteremia or in the prevention of metastatic infection of the joints, but of the four employed in this work, only one hastened recovery from the intradermal lesions and exerted an appreciable influence upon the streptococci contained in them.

#### DISCUSSION

The chief value of these experimental lesions is that the intradermal one offers, in a suitable laboratory animal, a local lesion easily produced and observed and in many respects resembling streptococcus cellulitis of human beings, while the combined intradermal and intravenous inoculation provides a severe infection with bacteremia and metastatic infection of the joints. Both appear to offer satisfactory lesions for the experimental study of biologic and chemotherapeutic compounds. Not only may one make fairly accurate clinical observations of the local lesion along with associated temperature and leucocytic changes, but more importantly, the possible effect of therapeutic agents upon the streptococcus contained in the local lesions as well as those in the blood and joint lesions.

#### SUMMARY

1 The intradermal inoculation of adult rabbits with virulent hemolytic strains of Group A streptococcus gives rise to a local lesion and a sequence of events having an analogy to streptococcus cellulitis of human beings.

2 Combined intradermal and intravenous inoculation provides a severe infection with bacteremia and metastatic infection of the joints.

3 These symptom complexes have been described in detail, particularly as to the development of the local lesion, the temperature reactions, the leucocytic changes, the persistence of streptococci in the lesions, and the bacteremia.

4 Rabbits invariably recover from the local infection alone, but the majority of untreated animals finally succumb to the combined intradermal and intravenous infection.

5 These experimental lesions appear satisfactory for therapeutic investigations.

#### REFERENCES

- 1 Kolmer, J. A., Ruiziss, G. W., and Rule, A. M. The Chemotherapy of Streptococcus Infections of Mice With Special Reference to Salicyl Compounds, *J. Pharmacol. Exper. Therap.* 43: 71, 1931.
- 2 Kolmer, J. A. Chemotherapy With Special Reference to the Treatment of Syphilis, Philadelphia, W. B. Saunders Company, p. 79.
- 3 Goodner, K. Experimental Intradermal Pneumococcus Infection in Rabbits. *J. Exper. Med.* 48: 1, 1928.
- 4 Kolmer, J. A. and Rule, A. M. Goodner's Intradermal Pneumococcus Infection of Rabbits, *J. Infect. Dis.* 57: 47, 1935.
- 5 Kolmer, J. A., Brown, H., and Ruiziss, G. W. The Chemotherapy of Experimental Streptococcus Infections of Rabbits with Special Reference to Pyridine Compounds and Sulfanilamide. *J. Pharmacol. & Exper. Therap.* (To be published.)

about the maximum of intensity, showed that the epidermis is not involved but that the corium is enormously infiltrated with polymorphonuclear leucocytes with small amounts of serofibrinous material. There is some congestion of blood vessels and lymphatics and the subcutaneous connective tissues are swollen and slightly edematous. In some instances the leucocytic infiltration and edema involve the superficial muscle layers. Sections have usually shown an abundance of streptococci in the exudates, some of which are intracellular.

*Lesion Cultures.*—Cultures of fluids aspirated from the local lesions twenty-hours after infection were invariably positive and daily cultures usually continued positive for 3 to 6 days and sometimes even when the lesions were

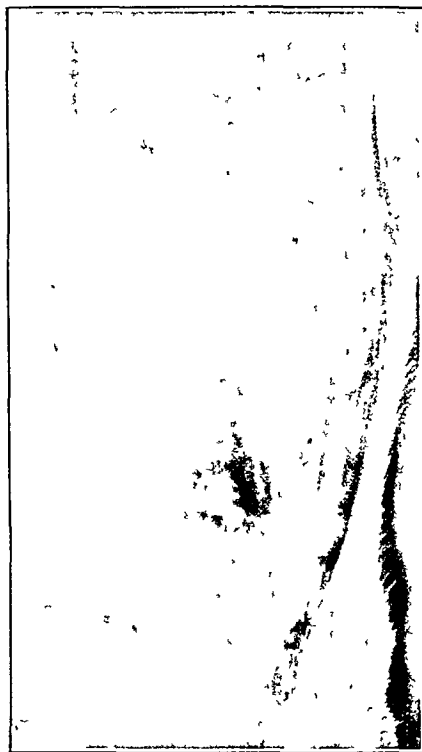


Fig. 1—A photograph of a focal lesion twenty-four hours after intradermal infection.

healed to +. Technical difficulties and healing sometimes prevented successful aspirations after the third or fourth day, but in general terms it appeared that streptococci tended to persist in the lesions until healing was far advanced and almost complete.

*Bactericemia.*—Blood cultures were only occasionally and irregularly positive in the course of the intradermal infection. In the majority of animals they were negative throughout. Occasionally positive cultures were found in animals receiving intravenous injections of new chemical compounds and especially in the case of those producing temporary exacerbation of the local lesions.

regulatory mechanism (Fig 1, *B*) A general decrease in temperature obtained with cocaine confirmed the findings with methylene blue

Anesthesia also substantially lowered body temperature in a chamber maintained at 76° F Fig 1, *C* shows the usual organ body constancy which remained during a decrease, maintained low level, and subsequent increase of body heat upon administration of sodium amytal in dosages suggested by Nicholas and Barron<sup>4</sup> Morphine sulphate injected subcutaneously had inconspicuous effects on the level of body heat but no effect upon the uniformity of visceral temperatures

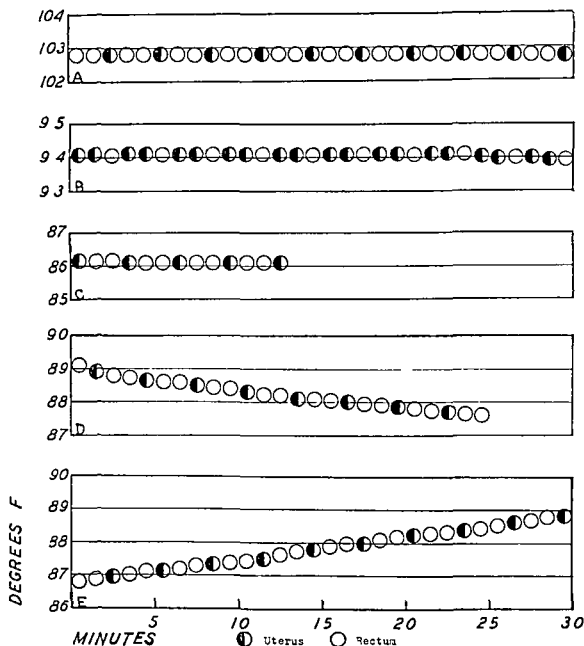


Fig 1—Agreement of uterine and body temperatures *A*, In hyperthermia. Fever induced by injection of yeast *B*, In drug induced lowering of body level of heat (injection of methylene blue) *C*, In anesthesia (sodium amytal) *D*, In hypothermia due to cold environment. Animal unanesthetized *E*, In the absence of blood flow. Dead animal placed in warm chamber

**3 Variance in the Body Heat Level Induced by Extremes of Environmental Temperature** After the physiologic changes which occur in the uterus<sup>2</sup> and body changes produced by drugs were found insufficient to alter the thermal equilibration of the body, more severe and marked changes in body temperature were produced

Hyperthermia was induced either by placing the rat directly in a chamber at 95° F, or by gradually increasing the usual (76° F) chamber warmth

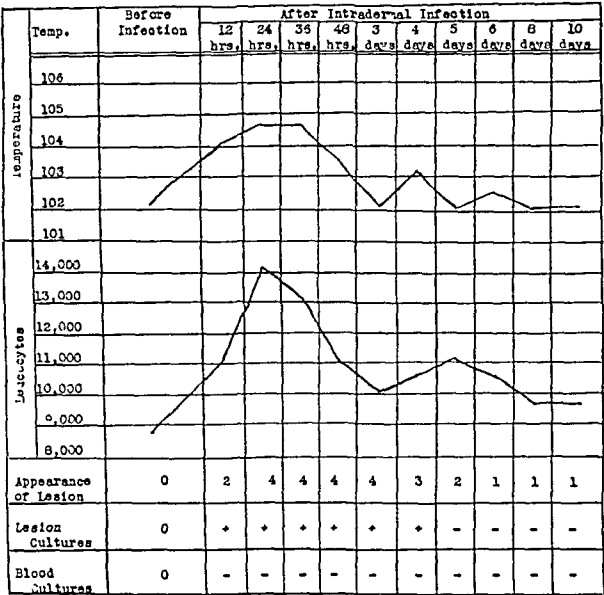


Chart 1.

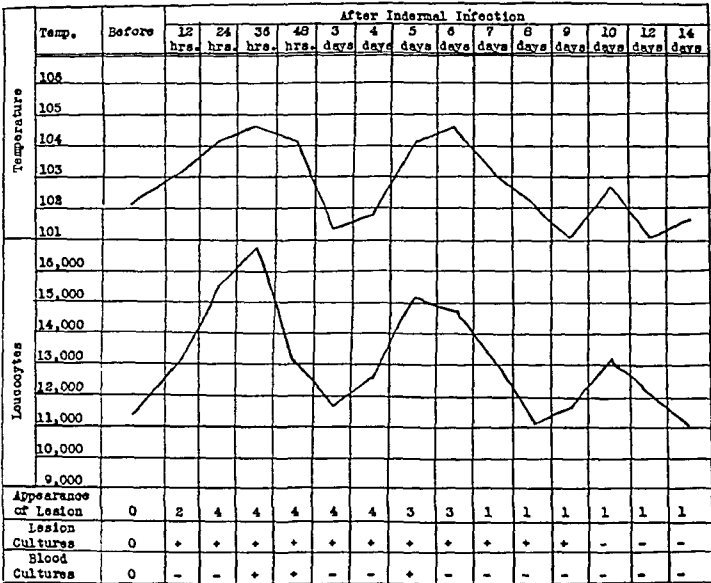


Chart 2.

in progress that there exists throughout the other abdominal organs an *internal integration of heat*, a homeostasis that remains undisturbed physiologically or pharmacologically. As it seems logical to assume that all internal parts do not possess the same metabolism or identical heat production, these findings of uniform abdominal warmth arouse interest as to the mode of integration of warmth from one part to another. In the presence of differential heat production, how is the integration of organ and system maintained?

In its totality, the temperature of the animal body is a resultant of the interplay between forces producing heat and forces dissipating this thermal energy from the body. In an environment lower than the body temperature, the following equation holds:

$$\text{Heat Production} - \text{Heat Elimination} = \text{Body Heat}$$

The physical means of heat elimination from the body surface are conduction, convection, radiation and evaporation. In the elimination of heat within the body, that is, equilibration of warmth from one internal part to another, radiation and evaporation may be disregarded. The following set of experiments were devised to test conduction and convection in the internal integration of heat:

- a. An organ with reduced blood flow
  1. A hypothermic state of reduced flow through the whole body
  2. A castrate state with reduced flow through the uterus
  3. Castrate animals in hypothermic conditions
- b. An organ with increased blood flow
  1. Hyperthermic states with increased blood flow through the whole body
  2. Hormonal stimulation, estrual and pregnant conditions with increased blood flow through the uterus
  3. Pregnant animal in hypothermic conditions
- c. Conduction by the tissues entirely apart from convection by blood flow
  1. Lack of blood flow through the organ
  2. Lack of blood flow through the whole body

In a hypothermic rat with a body heat level reduced to 56° F. the heart rate is irregular, weak and only 40 per minute as compared to 200 to 400 per minute at normal heat levels. The appearance of the rat is a pale gray, bordering upon cyanosis. The eyes are whitish pink, the red color fading away with the advancing state of hypothermia. Obviously, blood flow is markedly lessened, but the organ body uniformity of heat is not disturbed by the reduced thermal convection. The uterus of the castrate animal, despite lessened circulation through the organ, conforms to the body level of heat in a manner indistinguishable from the normal animal not only during normal conditions but also during hyperthermia, hypothermia, and rapid change in general body temperature.

Similarly, increased flow of blood through the organ (theelin injections, pregnancy) or through the body as a whole (hyperthermia)—or through both organ and body (hyperthermic animal during pregnancy)—does not affect the thermal integration of the abdominal part with the whole body. Sudden and complete elimination of uterine blood flow in the living unanesthetized

as long as one to five months. When arthritis was well developed the blood cultures were usually sterile thereafter.

The joints of the front and hind legs including the hips were those usually involved, with no regularity in the distribution of the lesions.

Among surviving animals the arthritis became suppurative with invariably positive joint cultures, but in no instance have we observed spontaneous discharge of pus.

Upon death, autopsy has frequently shown the presence of serofibrinous fluid in the pleural and peritoneal cavities, with positive cultures. No other gross lesions were found.

#### OBSERVATIONS ON SERUM THERAPY

Our main interests have been in chemotherapeutic studies with new synthetic chemical agents in the treatment of these experimental streptococcus infections,<sup>5</sup> but we have also tried some of the commercial antistreptococcus sera in the treatment of the focal lesion produced by intradermal infection.

The results of one of these experiments with two polyvalent concentrated antistreptococcus antitoxins and two whole sera are summarized in Table I. Erysipelas antitoxin was not employed.

TABLE I

EFFECT OF POLYVALENT ANTISTREPTOCOCCUS SERUMS UPON THE INTRADERMAL INFECTION

SERUMS	DOSE PER KILO (C.C.)	NUMBER DOSES*	LESIONS; DAYS								LESION CULTURES; DAYS							
			1	2	3	4	5	8	14	1	2	3	4	5	8	14		
(A) Concentrated	1.0	5	+	+	3	2	2	2	1	+	+	+	+	+	+	-	-	
(A) Concentrated	1.0	5	+	+	4	3	2	2	1	+	+	+	+	+	-	-	-	
(A) Concentrated	0.5	5	+	+	3	3	2	2	1	+	+	+	+	+	-	-	-	
(B) Concentrated	1.0	5	+	+	4	3	0			+	+	+	+	0				
(B) Concentrated	1.0	5	+	+	3	2	2	1	1	+	+	+	+	-	-	-	-	
(B) Concentrated	0.5	5	+	+	4	3	2	1	1	+	+	+	+	+	-	-	-	
(C) Whole	1.0	5	+	2	1	-	-	-	-	+	-	-	-	-	-	-	-	
(C) Whole	1.0	5	+	2	1	-	-	-	-	+	-	-	-	-	-	-	-	
(D) Whole	5.0	5	+	+	3	2	2	2	1	+	+	+	+	+	+	-	-	
(D) Whole	2.0	5	+	+	4	3	1	1	1	+	+	+	+	+	-	-	-	
(D) Whole	1.0	5	+	+	4	3	2	1	1	+	+	+	+	+	-	-	-	
Control	-	-	+	+	4	4	3	2	1	+	+	+	+	+	+	-	-	
Control	-	-	+	+	4	2	2	1	1	+	+	+	+	+	-	-	-	
Control	-	-	+	+	4	4	4	3	3	+	+	+	+	+	+	+	-	

\*By intravenous injection. First dose given four hours after intradermal infection, followed by four additional doses at daily intervals.

Each serum was given by intravenous injection, the first dose being administered four hours after intradermal infection when the local lesions were already developing, followed by four additional doses at daily intervals. The amounts administered varied from 0.5 to 5.0 c.c. per kilogram of weight, as shown in the table.

No appreciable effects were observed on the clinical course of the local lesions or upon the lesion cultures by three of these serums (A, B and D) but serum C appeared to exert a definite therapeutic influence since the local lesion rapidly regressed along with apparent rapid destruction of streptococci in the lesions (negative cultures). Under the circumstances, it may be in-

TABLE I

CASE	BLOOD SERUM				URINE				
	VAN DEN BERGH MG. %	LITMA FILTRATE	DIALYSATE		COLOR	VAN DEN BERGH 10 mg. %	GMBLIN (HNO <sub>3</sub> ) +	LITMUS PATE Normal urine color Negative Gmelin and diazo	DIALYSATE COLLOIDION No color 12 hours
			COLLOIDION	CEPHALAN					
S	24.0	Colorless	Colorless 1-4 hours	Not done	Highly pigmented		+	Normal urine color Negative Gmelin and diazo	No color 12 hours
L	19.5	Colorless	Colorless 4-24 hours	Colorless 24 hours	None obtained				
P	14.0	Colorless	No color 8 hours slight yellow tinge 24 hours Negative HNO and diazo	Not done	Very dirty		+	Normal urine color Negative HNO <sub>3</sub> and diazo	No color 12 hours
H	15.8	Colorless		Not done	Highly pigmented	Qualitative positive	+	Normal urine color Negative HNO <sub>3</sub> and diazo	
R	17.4	Colorless		Not done	Highly pigmented		+	Normal urine color Negative HNO <sub>3</sub> and diazo	

# THERMOREGULATION AMONG THE VISCERA WITH DESCRIPTION OF A MEANS OF PRODUCING HYPOTHERMIA IN UNANESTHETIZED ANIMALS\*

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BY PREVIOUSLY implanted thermocouples temperature determinations can be made of internal organs in intact and unanesthetized animals.<sup>1</sup> These reveal a thermal uniformity of abdominal organs.<sup>1, 2</sup> The uterus of the rat remains in agreement with the body level of heat throughout the physiologic changes undergone in estrual rhythms, pregnancy, parturition, lactation, and involution. Similarly, the more rapid changes induced by injection of theelin and adrenalin do not affect the common organ-body level of heat.

These data represent the capacity of the uterus to respond to severe and rapid changes of the body level of heat by pharmacologic and physical means. It presents an opportunity to study the parts played by direct tissue conduction and by blood convection in the maintenance of a thermal homeostasis among the abdominal organs.

*Results.—Variance in the body heat level induced by drugs.*

1. *Elevation by Yeast and Dinitrophenol:* Subcutaneous injection of 10 c.c. per kilo of a 15 per cent solution of Northwestern yeast produces a steady febrile level in the rat which lasts eight to sixteen hours. Fig. 1, A exemplifies the uniformity of uterine and body temperatures during the rise, high level, and fall of yeast fever in 5 rats.

A dosage of 25 gm. per kilo was used for the production of dinitrophenol fever in another series of 5 rats. This dosage approaches the lethal figure suggested by Tainter, Bergstrom and Cutting,<sup>3</sup> but the elimination of movement by previous acclimation to immobilization cages may have tended to prevent a larger degree of rise. A thermal agreement similar to that in yeast fever is seen between uterus and body through the rise, the high level, and the fall of the dinitrophenol fever. Acetanilid acted as an antipyretic to lower the body temperature in 3 animals without disturbing the organ-body relationship.

2. *Lowering of the Level in the Rat by Methylene Blue, Cocaine, and Anesthetics:* The rat occupies a distinctive position among laboratory animals in its peculiar reaction to certain drugs, such as cocaine and methylene blue, which induce febrile conditions in the cat and dog but serve to lower body temperature in the rat. Methylene blue of M/100 solution in dosages of 10 c.c. per kilo caused a fall in the body heat level of as much as 4° F., within an hour. As in the results obtained with pyretics, a thermal equilibrium between the uterus and the body was maintained despite disturbances of the heat

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## THE SO CALLED "FIRST ACID CHANGE" IN FRESHLY SHED BLOOD\*

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A CHANGE of pH of from 0.02 to 0.095 units occurring in blood freshly removed from the body was described by Havard and Kerridge (1929), using the glass electrode, and confirmed by Laug (1931) by hydrogen and quinhydrone electrode measurements. In a study by Platt and Dickinson (1933) of the conditions required for accurate measurements by the glass electrode, it was found that temperature differences arising out of manipulations or due to factors in the design of the electrode system could give rise to potential differences which might be interpreted as changes, in the solutions examined, of hydrogen ion concentration—acid or alkaline according to conditions. These results, to us, afforded an explanation of the "acid change" of Havard and Kerridge, and it was further stated that no experimental evidence had been obtained of an acid change in blood of the type described by these writers. Other observations (Dickinson, Havard and Platt, 1933), in which some of the measurements were made by Havard, using the Stadie form of glass electrode, confirmed this view. It was also shown that, with blood on one side of the membrane, apparent acid or alkaline changes follow temperature differences according as a phosphate buffer solution of pH 7.0 or N/10 HCl was used on the other side of the membrane.

The first acid change was investigated by Laug (1934), who, by eliminating the possible artefact due to temperature differences, maintains that there is an acid shift in freshly shed blood, and that this acidity is probably the result of glycolysis preventable by the addition of potassium fluoride in comparatively large amounts. Recently Ferguson and DuBois (1936) have taken up this question. They state that Laug "gave good arguments against the possibility that the acid shift was a mere 'temperature artefact' as suggested by Platt and Dickinson." They feel that the artefacts discussed by Laug may be ruled out in their experiments. "The present data," they write, "agree with those of Laug and of Havard and Kerridge in identifying a true 'acid shift' in shed blood."

Sifting the evidence, in view of the statements of Ferguson and DuBois, reveals, however, several inconsistencies in the published data and the conclusions drawn from them. The present situation has arisen mainly out of confusion of the "first acid change" as originally defined and the acidity developed in blood as a result of glycolysis. In this note the evidence which has accumulated in support of the contention of Platt and Dickinson that the first acid change was an artefact is presented and the extent to which changes in acidity occur in freshly shed blood is indicated.

The main criteria of the first acid change as described by Havard and Kerridge are that it is unaffected by sodium fluoride, that it takes place

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The uterine temperature agreed with the body level of heat during the hyperthermic rise, the maintained elevation and later the fall in body temperature.

Death intervenes at less than 10° F. above normal body temperature (approximately 109° F.). More conclusive data concerning uterine conformity to the body level of heat are obtained from the hypothermic states. The rodent has been found in these experiments to have an exceedingly rapid heat loss in a moderately cool environment (35° F.) without the use of anesthesia or other means of destroying body heat regulation as is necessary for rapid and pronounced heat loss in the cat, dog, and other animals. The loss of heat is abrupt and not effectively interrupted by shivering or other cold counteractant. Rats spontaneously recover from one- to two-hour exposures even though the temperature of the body decreases to the low level of 60° to 54° F., as ascertained by a deep rectal thermocouple or from readings of various internal organs. Upon removal from the cold chamber, the animal spontaneously begins the resumption of the usual level of body heat. In such hypothermic conditions the thermal relationship of the organs to the rest of the body was examined for any trace of (1) a lag in organic temperature above the general body level during heat loss, or (2) any persistently higher organic temperature at low levels of body heat, which might be taken as an indication of a distinctive source of heat production.

Care was taken in the interpretation of readings when the chamber was cold enough to produce a particularly rapid loss of body heat, for in these conditions the rectal thermocouple may register higher than the thermocouple implanted in the uterus, due to the thermal inertia of the larger mass of the rectal thermocouple which opposes any rapid change in heat level.\*

The data obtained from extreme variations of the body heat level in hypothermia confirm the unbroken uniformity of uterine and body temperatures which was observed in hyperthermia and in procedures that had induced a lesser degree of change in the body level of heat. Fig. 1, *D* shows similar uterine and colonic readings in the unanesthetized rat at 66° F.

Moreover, this conformity during extreme hypothermia was never seen to vary with the amount of blood flowing through the uterus. A striking illustration of this is seen in rats subjected to hypothermia during various stages of the estrual cycle, pregnancy, and finally in the castrate condition—with an exact thermal agreement of organ and body despite great differences in uterine blood flow combined with severe changes in body temperature.

#### DISCUSSION

The uterus of the rat conforms to the general level of body heat during (1) the extreme physiologic changes that occur in the organ as in pregnancy and in parturition, and (2) changes in the level of body heat which affect the uterus secondarily. Further evidence will be brought forward in work now

\*The testing of this theory lay in the suturing of a thermocouple from an internal abdominal approach to the intestine at a site identical with that of the rectally introduced thermocouple. Presumably, the two should register the same unless the larger mass of the rectal thermocouple causes a lag in rapid changes of body temperature. The 2 thermocouples recorded identically at any continued level of body heat and through ordinary cooling. In rapid falls, however, the rectally inserted lead lagged behind as much as a whole degree Fahrenheit.

but with proper temperature control and precautions to prevent loss of  $\text{CO}_2$  yield values for the pH of blood which are within the accepted limits of accuracy for the measurement of pH in general (i.e., 0.01 to 0.02 pH units)

## REFERENCES

- 1 Dickinson, S, and Havard, R E The Reaction of the Arterial Blood in Cancer, *Brit J Exper Path* 14 394, 1933
- 2 Dickinson, S, Havard, R E, and Platt, B S The Measurement of the Hydrogen Ion Concentration of Blood by the Glass Electrode, *J Physiol* 78 23, 1933
- 3 Ferguson, J H, and DuBois, D Observations on the pH of Clotting and Citrated Blood, *J LAB & CLIN MED* 21 603, 1936
- 4 Harris, I, Rubin, E L, and Shutt, W J Modifications in the Use of the Glass Electrode for the Determination of the pH of Venous Blood, *J Physiol* 81 147, 1934
- 5 Haugaard, G, and Lundsteen, E The Measurement of pH in Blood by Means of the Glass Electrode, *Compt rend Carlsberg* 20 1, 1936
- 6 Havard, R E, and Kerridge, P T An Immediate Acid Change in Shed Blood, *Biochem J* 23 600, 1929
- 7 Laug, E P The Application of the Quinhydrone Electrode to the Determination of the pH of Serum and Plasma, *J Biol Chem* 88 551, 1930
- 8 Laug, E P A Reinvestigation of the Phenomenon of a First Acid Change in Whole Blood, *J Biol Chem* 106 161, 1934
- 9 Platt, B S, and Dickinson, S The Technique of Electrode Measurements, *Biochem J* 27 1069, 1933
- 10 Yoshimura, H On the Acid Change in Shed Blood, *J Biochem* 21 335, 1935

## SUPRARENAL TUMOR WITH PAROXYSMAL HYPERTENSION\*

## CASE REPORT

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IN RECENT years there have been reported several cases of tumor of the adrenal medulla or other chromaffin tissues, associated with paroxysmal attacks of hypertension and other manifestations of sympatheticotonia. The first classical description was by L'abbe, Tinel and Doumer, in 1922. C H Mayo, in 1927, reported the successful removal of one of these tumors with clinical cure of the patient. Belt and Powell have recently presented a case with a review of the literature. I wish to present another case of this type.

## CASE REPORT

The patient was a girl twelve years old, when first seen, in March. Her illness began with what was said to be mumps of the ovaries four years previously. At that time she had much pain in the lower abdominal quadrants and was in bed for a few weeks. After that, the patient developed a ravenous appetite, profuse sweating, and purplish discoloration of the skin of the hands and feet. Two years ago, a basal metabolic rate determination was said to have given a figure of plus 60. For the past year or so there had been attacks of severe pectoral pain, accompanied by profuse sweating and discoloration of the skin. The severity and frequency of the attacks had been increasing.

When first seen, the patient looked to be of about the stated age, and was quite well developed and nourished. There was beginning development of the breasts and a small

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## METHODS

The ultrafiltrations were done through collodion sacs made according to Greenberg and Gunther;<sup>2</sup> and according to the method previously described by us.<sup>3</sup> The purified bilirubin was prepared and the serum bilirubin was determined by the van den Bergh method as modified by Gibson and Goodrich,<sup>4</sup> Thannhauser and Anderson<sup>5</sup> and Newman.<sup>6</sup> The cellophane employed was the DuPont product of tubular design and about three-fourths inch in diameter. The solution to be dialyzed was placed in a loop of this and both ends were tied up out of the fluid, against which the serum or urine was being dialyzed. In some instance (see Table I), the serum and urine and bilirubin solutions were ultrafiltered in the collodion sacs, following which they were dialyzed against distilled water in the same sac. The volume of the distilled water against which the serums and urines were dialyzed was 25 c.c. in each instance. Eight to 10 c.c. of serum or urine were employed in each ultrafiltration or dialysis.

An "artificial" jaundiced serum of high bilirubin content was prepared by dissolving gallbladder bile in pooled normal serums. This was studied in the same manner as the above.

## RESULTS

Table I shows the results of our studies of the serum and urine in 5 cases of jaundice with bilirubinuria, all showing van den Bergh reactions of the direct immediate type.

No bilirubin went through the collodion membrane in any of the 5 cases, although the bilirubin in the blood ranged from 15.8 to 24 mg. per cent and there was bilirubin in the urine. Furthermore, bilirubin which had passed through the kidney would not filter through the collodion membrane, demonstrating that the filtration through the kidney and through collodion membranes is not comparable.

Serum with the bilirubin augmented by the addition of gallbladder bile until the concentration of the bilirubin was 34.8, likewise gave an ultrafiltrate containing no bilirubin.

It is also noteworthy that water solutions of purified bilirubin gave a clear filtrate although no protein was present to "bind" the bilirubin and prevent its passage through the filter.

The results on solutions of the sodium salt of bilirubin and on "artificial" jaundiced serum are tabulated in Table II.

## COMMENT

We are unable to explain the lack of agreement between our results and those of Hoover and Blankenhorn and of Leschke in regard to the dialysis of bilirubin from icteric serum obtained from patients who showed hyperbilirubinemia and bilirubinuria. The pore size of their membranes may not have been carefully controlled and some of these may have had pores large enough to allow bilirubin to go through. We have found that if the membranes allowed traces of protein to pass, bilirubin would also go through.

The results of the present experiments clearly indicate that bilirubin of blood serum and urine in obstructive jaundice, bilirubin in gallbladder bile,

One fourth cubic centimeter of adrenalin was given, and in a few minutes there was much improvement. The next day there was another severe attack followed by shock. Adrenalin was again administered, but before other shock therapy could be instituted, the patient died, the respiration continuing for a few seconds after the heart had stopped.

*Autopsy*—The livid discoloration was replaced by the usual postmortem pallor. There was no free fluid in the thoracic cavity and the lungs showed no signs of pulmonary edema or other abnormality. The heart appeared normal in size. Examination of the endocardium and valves revealed no abnormalities. The myocardium appeared normal on cut section. On opening the coronary arteries, there was found no evidence of arteriosclerosis other than a few small scattered atheromatous plaques. The aorta also contained only a few similar plaques. On exploring the left renal area there was found a tumor about the size of a hen's egg. This was situated anterior to and above the kidney retroperitoneally, with the tail of the pancreas around it. The adrenal gland was intimately associated with the tumor, about one half of its substance projecting from its surface. The mass shelled out quite readily, but there was some adherence to the peritoneum. The tumor was rather soft, and on opening it, was found to be composed of pliable glandular tissue with some cystic degeneration and gelatinous formation in the center. The right adrenal gland appeared grossly normal. Both kidneys appeared normal, as did the stomach, intestine, liver, and spleen. There were many enlarged lymph glands in the mesentery.

The specimen was sent to Dr. Donald Beaver of the Department of Pathology of the Mayo Clinic, who reported the tumor to be composed of adrenal medullary tissue, a pheochromocytoma.

#### COMMENT

The case is interesting from many viewpoints. Could surgical aid have been secured early enough, the patient might have been cured. While the operation is a hazardous one, as has been found in previous cases, the tumor showed no signs of malignancy and its removal would have induced permanent relief from the attacks.

The clinical picture was quite like the other reported cases, but the death was different. Of the other cases that terminated fatally, that of L'abbe, Tinel and Doumer died of pulmonary edema. Those of Rabin and Vaquez, Danzelot and Gerandel died in coma and that of L'abbe, Violle and Azerod died of cerebral hemorrhage. In the case reported here the patient was perfectly conscious a few minutes before death, and at autopsy there were found no signs of pulmonary edema. Death was apparently due to shock, or a condition closely simulating it.

In all of the cases in which the tumors were removed, severe shock was a postoperative complication and the patient of Belt and Powell died of postoperative shock. This might lead one to hazard a theory. It would appear that the body has some mechanism of defense against the abnormally large amounts of adrenalin produced by the tumor. Perhaps this defense mechanism, be it an "anti hormone" as suggested by Collip, or the breaking down of adrenalin by the liver or other organs, becomes suddenly too powerful when the tumor is removed, with the development of shock (antithesis of the condition occurring in the attacks).

Why, in all cases reported, the syndrome should be of such a paroxysmal nature is problematical. In the Porters' case the patient could produce attacks at will by twisting the body into certain positions. It would appear that mechanical pressure on the tumor might have caused the expulsion of adrenalin into the circulation. In the case above reported, as well as in several of the previous

TABLE II

	VAN DEN BERGH MG. %	ULTRAFILTRATE	DIALYSATE	
			COLLODION	CELLOPHANE
"Artificial serum"	34.8	Colorless	Colorless 20 hours	Colorless 20 hours
Bilirubin Solution A	2.0	Colorless		
Bilirubin Solution B	8.8	Colorless		
Equal parts of normal serum and Bilirubin B	Approx. 5.0	Colorless		

and purified bilirubin in aqueous and serum solutions do not ultrafilter through collodion membranes of the type described and at the pressure indicated. Likewise, it does not dialyze through the cellophane membrane described.

It is well known that the spinal fluid of patients with marked hyperbilirubinemia and bilirubinuria does not contain bilirubin. Our own experience confirms this.

Our experiments show that serum bilirubin of patients with marked hyperbilirubinemia and bilirubinuria is entirely different in its filtrability through the human glomerulus and through collodion membranes. Its capacity to pass the spinal fluid barrier and through collodion and cellophane membranes appears to be the same.

#### CONCLUSIONS

Neither the bilirubin of the serum nor that of the urine of patients with obstructive jaundice can be ultrafiltered or dialyzed under the experimental conditions described. This is also true of bilirubin which has passed through the liver cells.

It appears, therefore, that the previous report of the "unvarying consistency in the behavior of bilirubin toward the collodion dialyzer and toward the renal filter" is untenable.

#### REFERENCES

1. Hoover, C. F., and Blankenhorn, M. A.: Dissociated Jaundice, *Arch. Int. Med.* 18: 289, 1916.
2. Greenberg, D. M., and Gunther, L. On Determination of Diffusible and Non-diffusible Serum Calcium, *J. Biol. Chem.* 85: 491, 1930.
3. Gregory, R., and Andersch, M.: Filterable Calcium of Blood Serum; Comparison of Filterable Calcium of Serum and Total Calcium of Cerebrospinal Fluid in Normal, Hyperparathyroid, and Hypoparathyroid States, *Am. J. M. Sc.* 191: 263, 1936.
4. Gibson, R. B., and Goodrich, G. E.: Determination of Plasma Bilirubin; Modified van den Bergh Procedure, *Proc. Soc. Exper. Biol. & Med.* 31: 413, 1934.
5. Thannhauser, J. A., and Anderson, E.: Methodik der quantitativen Bilirubin Bestimmung im Menschlichen Serum, *Deutsche Arch. f. Klin. Med.* 137: 179, 1921.
6. Newman, C. E.: Bilirubin and van den Bergh Reaction, *Brit. J. Exper. Path.* 9: 112, 1928.
7. Blankenhorn, M. A.: Acholuric Jaundice, *Arch. Int. Med.* 27: 131, 1921.
8. Leschke, E.: Fortsetzung der Aussprache über Ikterus, *Berl. Klin. Wechnschr.* 58: 848, 1921.
9. Barron, E. S. G.: Bilirubinemia, *Medicine* 10: 77, 1931.

## A SUPPORTING, ELASTIC BELT FOR USE IN ABDOMINAL OBESITY (POSTURAL SYNDROME)\*

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MANY belts have been devised and are used routinely for abdominal support. Their main purpose is to correct either obesity or ptosis of the abdomen by lifting and holding in the abdominal wall. They accomplish this by supplementing or replacing the abdominal musculature which has either lost its tone or, more usually, has become flabby and no longer functions properly. These belts are constructed in many ways, but all have similar materials and serve the same purpose. We have a collection of inadequate belts donated by patients, and these show great variation in type.

We have devised a belt for use on patients suffering from symptoms due to the effects of a pendulous abdomen. Obesity, most frequently generalized but occasionally limited to the abdomen, is usually the precipitating factor. The result, when the condition has reached the point where the abdominal muscles can no longer sustain the added weight, is a heavy, pendulous belly. The condition frequently results in a train of symptoms which we have termed the "postural syndrome". There is no doubt that any individual with abdominal ptosis, whatever its cause, will have symptoms and signs when the condition has been present long enough. The correction of the condition in these patients depends upon loss of weight, restoration of muscular function, and postural exercises. A proper supporting belt is a distinct aid in treatment, in the early months especially.

The belt that we are presenting embodies several new principles. As a whole it is not unusual, or very different from the many we have seen. However, it is constructed with the physiologic function of the abdominal wall in mind, and is designed to supplement and aid the ventral muscles rather than to replace them. A tightly wound cloth binder would support the abdomen more efficiently but would not permit mobility necessary for respiration.

Fig 1, C shows the belt in place on a patient. Fig 1, B shows some of the details of construction. The supporter is made of coutil, pekín stripe cloth and elastic goring, it is fastened on by skate buckles and hooks.

The front sections and the back section are of double thickness. The outer layer is of high quality coutil, the inner of pekín stripe cloth. The only difference between the two is in the softer and finer quality of the pekín stripe material which, being next to the skin, prevents chafing. Both sections are tailored or fashioned. There are three double stays in the back section with a seam at each, which permits fashioning the supporter to fit the contours of the individual patient. The back section is  $8\frac{1}{2}$  inches in height, and the stays are of whale

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within about six minutes at 38° C., and that it has a mean value of 0.05 pH. Laug (1934) himself states that well-defined acid changes proved to be due to nothing more than mere temperature artefacts traceable to warming up. The values he gives for the acid shift, for example, range from 0.05 pH units in about thirty-five minutes (as shown in his Fig. 1) to an average of 0.015 pH in twenty minutes for human blood as shown in Table II. The results of Ferguson and DuBois show (as shown in their Table I) only very slight changes in acidity and in one case an alkaline change in the course of twenty to thirty minutes during the clotting of rabbit's blood. The experiments in which significant changes in pH were observed were of several hours' duration and were carried out without the addition of fluoride.

Yoshimura (1935), in two of three experiments with fluorized human blood, found only a slow alkaline change beginning one hour after shedding; in the third experiment an acid change set in after about six minutes, continued for two hours, and was followed by an alkaline drift. He concludes from his work that the existence of the first acid change cannot be confirmed; that such acid change as is found in fresh blood is continuous and is due to glycolysis which is inhibited by sodium fluoride. This substance is, however, held responsible for an acid change due to an effect on the corpuscular membrane.

Harris, Rubin, and Shutt (1934) also agree with our finding that there is no acid change of the type found by Havard and Kerridge.

Recent careful work by Haugeard and Lundsteen (1936), using an electrode arrangement similar to that of Platt and Dickinson, is concerned more particularly with the relation between the change of pH of blood immediately after its removal from the body and the decrease of sugar. They have shown that the development of acidity follows closely the disappearance of sugar. During the first ten minutes after shedding, the change is no more than 0.006 pH. So small is the change of pH in their experiments that they consider the error of measurements made within about five minutes of obtaining the blood as being within the limits of experimental error for pH measurements. They appear to dispense entirely with anti-enzymatic substances.

The original experiments we made on blood were carried out in the presence of sodium fluoride and potassium oxalate. Not more than 10 c.c. of blood were withdrawn into a syringe containing 1 c.c. of 3 per cent potassium oxalate and 2 per cent sodium fluoride (neutralized); the same technique is described in detail by Dickinson and Havard (1933), who used it to determine the pH of arterial blood in cancer.

It must be conceded from the evidence here presented that there is no "first acid change" in freshly shed blood. The change originally described must have been a mere "temperature artefact." In the absence of fluoride, however, an acid change occurs which is part of a continuous glycolytic process. Even this reaction, according to the majority of workers, does not so rapidly result in alterations of acidity of the magnitude described by Havard and Kerridge. Careful examination of the published data goes to show that measurements made within five minutes of withdrawal from the body, without the addition of anticoagulants or anti-enzymatic substances,

The elastic lateral sections, Fig 1, *C* (3), each are made of two pieces of firm elastic going 6 inches wide and approximately 7 inches long, placed one above the other. The two pieces overlap about 2 inches in the center, giving greater support to the front center. The lower piece of elastic on each side is set diagonally downward toward the front, exerting therefore an upward lift rather than a straight posterior pull.

The uplift strap, Fig 1, *C* (4), is a combination of elastic and non-elastic material. The front forked section consists of firm elastic webbing  $1\frac{1}{2}$  inches wide. The latter extends to a buckle located toward the top of the suprapubic just back of the hips, allowing tightening or loosening of the strap as desired. The uplift straps, being placed diagonally, exert a supporting rather than a suppressing pressure on the lower abdomen.

The belt is fastened in front by means of six skate buckles and hooks (Fig 1, *B* (2)). The buckles are slipped over the hooks with the straps loose, each strap then being individually tightened to the desired degree. The extra length of strap is held up by slots at the origin, rather than allowed to dangle.

Fig 1, *A* shows the measurements required in making up a belt when the patient is not able to be fitted personally. The measurement for the upper edge of the belt is taken around the body about 1 inch above the anterosuperior iliac crest of the ilium. The measurement for the middle of the belt should pass over the largest part of the abdomen. The measurement for the lower edge should follow the lowest line of the abdomen in front, and cross the spine in back three to four inches below the upper measurement.

The dimensions of a size 40 belt are approximately as follows:

1 *Back*  $16\frac{1}{2}$  inches long at the top and 18 inches at the bottom, made of three double pieces of whalebone  $4\frac{1}{2}$  inches apart. Height is  $8\frac{1}{2}$  inches.

2 *Elastic side sections* 6 inches long, made of two pieces of elastic going 5 inches wide with  $2\frac{1}{2}$  inches' overlap, giving a height of 8 inches.

3 *Uplift strap*, the elastic arising at the bottom in front. 5 inches long, continued by a web strap  $6\frac{1}{2}$  inches long, passing through a tightening buckle inserted into the beginning of the back piece 2 inches below the top.

4 *Front sections*  $2\frac{1}{2}$  inches wide and  $9\frac{1}{2}$  inches high, the tongue  $1\frac{1}{2}$  inches long.

It should be stated that these measurements do not designate the construction necessary for proper fitting to the body, nor do they take into account the extra length required for seams, etc. They were taken from a finished product, laid flat.

Patients are taught to put on the belt before arising in the morning, preferably outside the undershirt. If a union suit is worn, the belt can be put on underneath it over a thin garment. The patient should be in the supine position when putting on the belt, and the belt should be tightened from below up. This is important in order to move the abdominal fat and viscera upward, rather than to compress it in the lower abdomen.

The unusual feature of the belt is the width and height of the elastic lateral sections, particularly the width. This is obtained by making the front narrow

amount of pubic hair. Menstruation had not occurred. The skin of the lower arms and legs was thickened and roughened and had a dusky red hue. The face was also affected in this way, but in a lesser degree. The pupils were equal and of medium size, and reacted promptly to light. The thyroid gland was normal to palpation. Over the cardiac apex could be heard a rather harsh systolic murmur, which was not transmitted. There was no cardiac enlargement to percussion. There was slight peripheral arteriosclerosis, as revealed by palpation of the brachial arteries. Examination of the ocular fundi revealed cotton-wool exudates in both macular regions with narrowing and some tortuosity of the retinal arterioles. The optic disks were swollen one or two diopters. No fresh hemorrhages were seen. Otherwise the physical examination revealed nothing noteworthy.

The laboratory data were as follows: Hemoglobin 88 per cent (Dare), erythrocytes 5,400,000, and leucocytes 14,200. The differential count revealed 71 per cent polymorphonuclear leucocytes, 28 per cent lymphocytes, and 1 per cent monocytes. The urinalysis was negative except for a trace of albumin (voided specimen). The Kline and Kolmer tests were negative. The phenolsulphonephthalein excretion was 65 per cent two hours after its intravenous injection. The blood urea was 50 mg. Roentgenograms of the chest and kidney areas revealed no abnormalities.

During the time the patient was under observation, a few attacks were observed. At the onset there would be a feeling of generalized weakness. After a few minutes there would



Fig. 1.—Photograph of suprarrenal tumor removed at autopsy.

be a very painful sense of constriction in the substernal and precordial areas, the hands and feet would become blue and cold and there would be profuse generalized sweating. At the end of the attack there would be a frontal headache, tinnitus aurium, and often repeated vomiting. The duration of the attacks varied from a few minutes to two hours. The paroxysms would take place at any time of the day, but most often in the morning, shortly after breakfast. The patient was unable to induce attacks by twisting or bending the body, as was the case in the patient reported by Porter and Porter.

During the attack, the blood pressure rose to as high as 285 systolic and 230 diastolic, and the heart rate became very rapid. The pupils were widely dilated. The retinal arterioles were so narrowed as to be scarcely visible. A blood sugar determination was made in an attack and the figure was 153 mg. per cent. (This was a few minutes after breakfast.)

After some discussion with the parents of the patient, she was finally hospitalized on May 14 for operative exploration of the adrenal glands. At that time there was a fever, ranging from 98.4 to 100.0°, and there was continuous sweating. Operation was scheduled for the morning of May 16, but as the patient developed some pectoral pain and an elevation of blood pressure to 185 systolic and 130 diastolic, the operation was deferred. These minor attacks continued daily, necessitating further postponement of the operation. On May 19 there was a very severe attack followed by symptoms and signs of shock. The blood pressure dropped to 30 and the pupils became very small. The heart rate was approximately 200.

## THE TOXICITY OF MORPHINE SULPHATE AND THE PRESSOR EPISODES\*

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IN A previous paper (Nedzel<sup>1</sup>), it has been shown that the Straub sign and the minimal lethal dose of morphine sulphate in mice vary from day to day. The animals used in our experiments were approximately of the same age, were kept under same conditions and had the same diets, the morphine used was of the same lot and, in experiments with minimal lethal dose, it was given proportionally to the individual weight of the animals, though such varied only a few grams.

With the modern concept of variability in physiologic state of the organism, intimately connected with constant changes in atmospheric conditions (Peter sen<sup>2</sup>), we have to look in this direction in seeking the explanation of our findings †

The first group of experiments consisted in producing a Straub sign in mice by injecting a small dose of morphine sulphate (0.4 mg). The mice were kept on our standard diet (Nedzel<sup>1</sup>). For seven weeks (five days a week), ten mice received morphine injections daily.

Graph 1 presents the results obtained in these observations. The upper broken line designates the positive Straub sign in 9 or 10 mice obtained on the given date. On the left is shown time (in hours) of duration of the Straub sign from the moment of morphine injection. Below this broken line the wide, uneven, black line, with unshaded line in the center expresses the official temperature on given days. The upper margin gives daily maximum temperature, the lower, the minimum temperature. The middle unshaded line presents the mean temperature. The lower curve is a barograph, giving the official barometric pressure for the days on which the experiments were performed.

The duration of maximal reaction (Straub sign) with our dose of morphine should according to Hermann<sup>3</sup> be 2¾ hours.

In our findings we see great variations in the duration of the Straub sign. As the graph shows, this reaction on certain days becomes shortened greatly, and

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†A pressor episode or pressor crisis (Pal crisis) the ARS phase of Petersen. Hochspannungskrise of the Germans represents a period of relative sympathicotonia when with adrenal and pituitary preponderance both systolic and diastolic blood pressure are increased. At the same time there is usually an increase in the blood pH and a decrease in the CO<sub>2</sub> content. During this period there is a tendency to contraction of the smaller vessels and capillaries and with this a relative anoxia may exist in the various regions. Following this phase a corrective phase sets in which is initiated by the accumulation of acids produced by anoxia, by capillary active substances by an increase in thyroid secretion etc. Constant pendulation of pressor crests with corrective phases of vascular dilatation are commonly initiated by change in the atmospheric circulation in our latitude but, needless to state a multitude of intercurrent events (psychic physical activity, trauma, etc.) may bring about similar though usually transient episodes of this character.

ones, the attack would occur for no apparent reason. However, there was a great tendency for the attacks to occur shortly after meals and especially after breakfast. This would suggest that some of the digestive organs played a part in maintaining this hypothetical balance between the adrenals and some other organ.

Unfortunately the thyroid gland was not removed at autopsy. Among the symptoms presented by the patient, there were many that could have been interpreted as due to hyperthyroidism, as well as hypersuprarenalism. However, there was no stare, tremor, nor history of recent weight loss. Determination of the basal metabolic rate was not attempted, because the patient could not be gotten into a condition anything like "basal."

#### CONCLUSIONS

1. A case of suprarenal medullary tumor (pheochromocytoma) has been presented with autopsy findings.

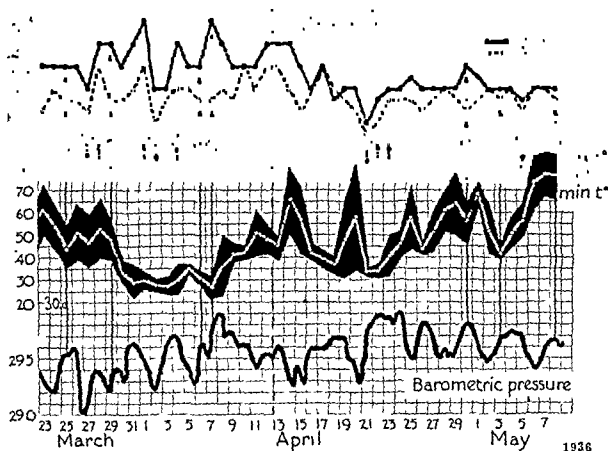
#### REFERENCES

1. Belt, A. E., and Powell, T. O.: Clinical Manifestations of the Chromaffin Cell Tumors Arising From the Suprarenal Medulla, *Surg. Gynec. Obst.* 59: 9, 1934.
2. Collip, J. B.: Inhibitory Hormones and the Principle of Inverse Response, *Ann. Int. Med.* 8: 10, 1934.
3. L'abbe, M., Tinel, and Doumer: Crises solaires et hypertension paroxystique en rapport avec une tumor surrenales, *Bull. et mém. Soc. med. d. hôp. de Paris* 46: 982, 1922.
4. L'abbe, M., Violle, P. L., and Azerod, E.: L'adenome medullaire surrenal avec hypertension paroxystique, *Presse méd.* 38: 553, 1930.
5. Mayo, C. H.: Paroxysmal Hypertension With Tumor of Retroperitoneal Nerve, *J. A. M. A.* 89: 1047, 1927.
6. Porter, M. F., and Porter, M. F., Jr.: Report of Case of Paroxysmal Hypertension Cured by Removal of Adrenal Tumor, *Surg. Gynec. Obst.* 50: 160, 1930.
7. Rabin, C. B.: Chromaffin Cell Tumor of Suprarenal Medulla (Pheochromocytoma), *Arch. Path.* 7: 226, 1929.
8. Vaquez, H., Danzelot, E., and Gerandel, E.: Le Surrenalome hypertensie, *Presse méd.* 37: 169, 1929.

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animals which were on water with added sodium citrate, their urine reaction being alkaline, and the interrupted line presents the findings in the group of mice that had water with hydrochloric acid and whose urine was constantly acid. In comparison with Graph 1, we have added here numbered arrows, pointing to the dates on which the prolonged or shortened Straub sign was observed simultaneously in both groups of animals. All other details are the same as on Graph 1.

We observe here, that the animals kept on water with hydrochloric acid showed, practically throughout the forty-seven days, a shortened duration of Straub sign. The vertical interrupted line drawn between 12 and 13 of April roughly divides all the observations in two parts. In the first part, where we see gradual, relatively slow changes in temperature, the duration of Straub



Graph 2.—The upper black line designates the positive Straub sign in animals on alkaline diet and the interrupted line presents the findings in the mice on an acid diet. All other details are the same as on Graph 1.

sign in both groups is longer than in the second part, and the mice with alkaline urine show a Straub sign considerably longer than that of the mice on hydrochloric acid. In the second part, where the temperature changes show greater variations, the length of the maximal Straub sign varies little in the two groups and is noticeably shorter in comparison with the first part of the experiment.

The vertical lines (which all, from 1 to 7, fall on the polar front) 1, 2, 3, 6, and 7 point out dates on which the alkaline mice withstood the pressor episodes and did not show any change in Straub sign, while the acid animals became less irritable and the duration of Straub sign in them was shortened. Vertical line 4 presents the date when there was a well-pronounced pressor episode and the acid animals retained their short duration of the Straub sign of the previous

bone. For conditions of extreme lordosis, the cloth section in back may be extended up to the twelfth thoracic vertebra, and have incorporated in it firm duraluminum or steel stays. The stays should not, however, be bent to fit the curve of the lumbar spine but should touch the body at only the upper and lower edges of the belt, allowing the middle to span or bridge the lordosed spine.

The left front section, Fig. 1, *C* (1), has the hooks attached to it. The right front section, Fig. 1, *B* (1), has attached to it the straps carrying the buckles. The height of the front from top to bottom varies somewhat according to the circumference. For patients 38 inches in circumference at the crest of the ilium,

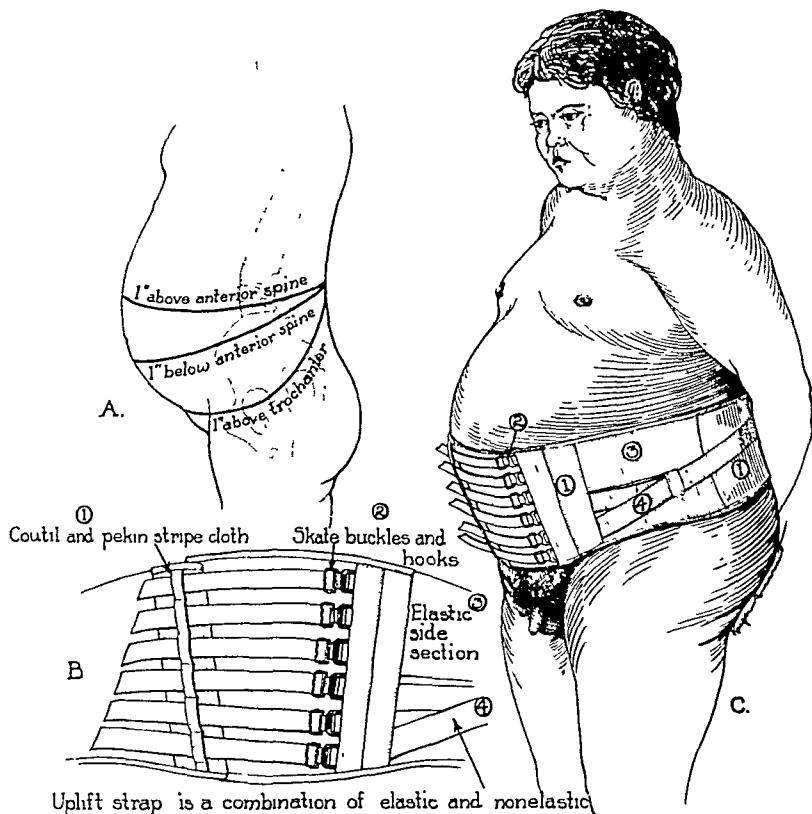


Fig. 1.

a height of 9 inches is usually most satisfactory; if the patient is exceptionally tall, a front height of 10 inches can be used to advantage; for patients of a circumference of 44 inches or more, a height of 10 to 12 inches is usually required. The left front section extends across the abdomen as a flap 6 inches long, padded between the two layers with strips of daisy cloth. This provides a soft base for the straps to lie on. The width of each half of the cloth section across the front is considerably less than in the usual supporter, being an average of  $2\frac{1}{2}$  inches wide. The total width of the nonelastic front is about 5 to 7 inches, but this varies with the tightness of the belt. The front flap slips under the right side of the belt as it is tightened.

prolonged duration of the Straub sign in both groups. On all these dates the pressor episodes have passed and either the temperature was rising or barometric pressure was declining.

The third series of experiments was on mice to which we have administered a minimal lethal dose (5 mg per 20 gm of weight) of morphine sulphate. The animals were divided into three groups. First group, controls, second, with sodium citrate in water, and third, hydrochloric acid in water. Daily, for thirty two consecutive days, to five mice of each group has been administered the mentioned dose of morphine sulphate. The findings are presented in Graph 3. The upper line presents the total deaths of mice of all three groups by days. The figures on the left show the number of dead animals of the total number of fifteen. The interrupted horizontal line at figure seven designates the average daily deaths, which has been seven. The three lines beneath express the daily death of mice from each group. The black line represents the deaths in the control group, the double line, alkaline group, and the cross hatched line, the acid group. The figures on the left show the number of mice that died. The lower lines give meteorologic data (temperature and barometric pressure) on the designated dates. The vertical lines point to peaks, where the average death rate of all three groups has been above the number of average daily deaths (seven).

The verticals 1, 2, 4, 7, 8, 9, 10, and 12 definitely connect the high death rate on corresponding dates with a pressor episode. Verticals 3 and 11 fall on the days with a sudden rise in temperature and falling barometer just after the pressor episode. Vertical lines 5 and 6 fall on slightly increased number of daily deaths, which coincide with a very slight falling barometric pressure, but here we had also a definitely falling temperature. The lowest death rates, as can be seen on the graph, coincide with moderate barometric pressure and high temperatures.

The lower lines, representing daily deaths in three groups of mice separately, point out unquestionably the daily variations in each group and these variations must be connected with the daily variations in meteorologic conditions, not only for vast clinical and experimental evidence (Peterson<sup>2</sup>), but also for lack of any other possible explanation. We consider that the number of animals used daily from each group (five) is inadequate for drawing definite and detailed conclusions. Additional experiments are to follow.

It is of interest to mention here observations carried out by Macht.<sup>4</sup> He found that there is a very definite influence of barometric changes and other meteorologic conditions on the potency of digitalis for cats. He points out that the difference in the toxicity is due to changes in the physiologic functions of the cats (his experimental animals) and more particularly to changes produced in the respiration and circulation by the fall in the barometer. He concludes that his observations on digitalis and some other drugs point to the fact that fluctuations in the barometric pressure and other changes in atmospheric conditions may play an important rôle in the action of various drugs.

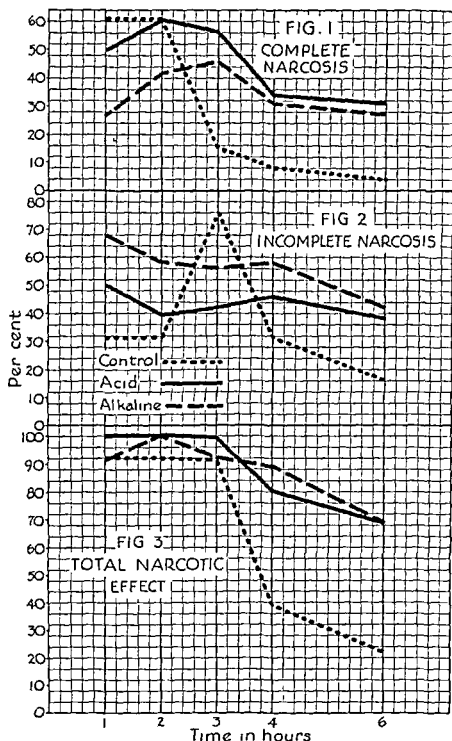
permitting the elastic side sections to extend farther forward on the abdomen where the elasticity is much more beneficial than if it were placed toward the back. This increased elasticity permits expansion of the belt during inspiration. This aids rather than suppresses abdominal breathing, and prevents limiting respiration to the thorax, as is seen in obese states naturally or where a firm, inelastic belt is applied. The increased elasticity also aids in expiration, and overcomes the prolonged expiratory period seen in those patients with depressed diaphragms due to abdominal ptosis or emphysema. The diaphragm more readily assumes the expiratory position, and is ready to descend with the next inspiration. There is no decrease in the supportive effect during the inspiratory expansion.

These belts have been made for us by the C. H. Hittenberger Co., 1103 Market St., San Francisco. We wish to express our appreciation to this firm for helpful criticism in the original designing of the belt, and for courtesy and care in fitting our patients.

#### REFERENCE

1. Kerr, Wm. J., and Lagen, John B.: The Postural Syndrome Related to Obesity Leading to Postural Emphysema and Cardiorespiratory Failure, *Ann. Int. Med.* 10: 569, 1936.

The observations were conducted on 78 rabbits, divided into three groups, each group consisting of 26 animals. Each group was kept from fifteen to twenty days on the special diets. In the first group (mixed diet) the animals were fed oats, carrots, alfalfa, cabbage, and water (our standard normal diet); the second group was fed oats and water; the third, carrots exclusively. By means of Folin's method the reaction of urine of the animals was tested daily.

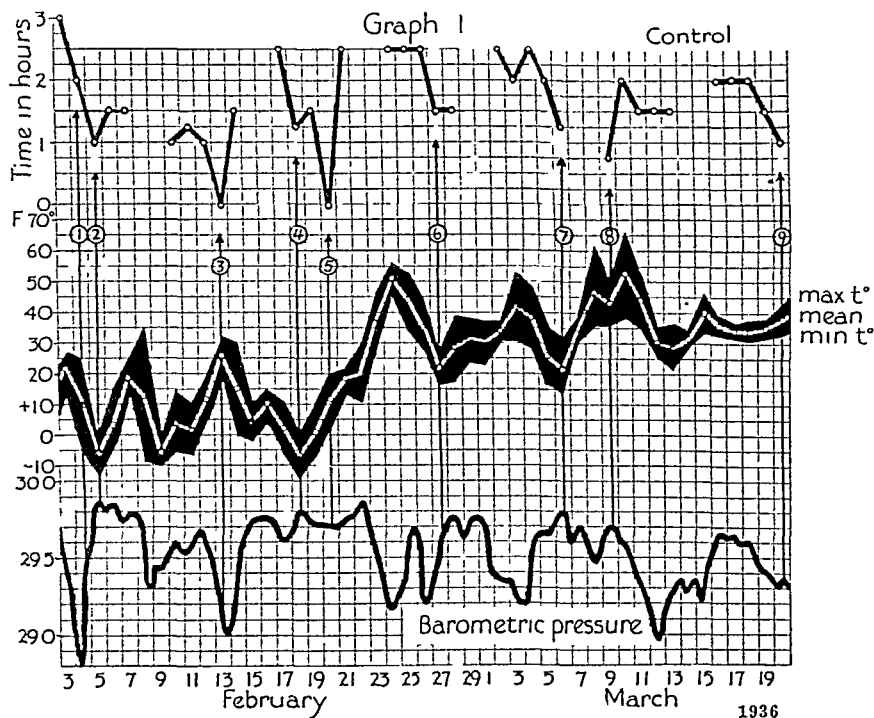


At the end of the first week the urine of the rabbits of the first group was slightly alkaline or acid, although generally more alkaline; the urine of the second group (oat diet) showed stable and distinct acid reaction (up to 90); and that of the third (carrot diet) group was persistently alkaline.

The experiments have been performed by injections of phenobarbital sodium into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of injection being about ten seconds. The dose of pheno-

if we draw vertical lines connecting the line representing the data of our findings with the lines of temperature and barometric pressure, we may establish a definite connection. These lines are numbered.

At lines 1 and 2 we record a drop in duration of a maximal Straub sign and this is connected with beginning of a pressor episode (polar front), the temperature drops and barometric pressure rises. At No. 3 we see somewhat the reverse condition, the temperature going up with a deep fall in barometric pressure. But if we look at the meteorographs of a few previous days, then we observe that the animals just went through a considerable pressor episode, are fatigued, which makes them unable to recover properly (Petersen<sup>2</sup>). We ob-



Graph 1.—The upper broken line designates the positive Straub sign in nine or ten mice obtained on the given date. On the left is shown time (in hours) of the duration of the Straub sign from the moment of morphine injection. The wide, uneven, black line expresses the official temperature (maximum, mean, and minimum) on given days. The lower curve is a barograph, showing the official barometric pressure for the days on which the experiments were performed. The figures below give dates on which experiments were done.

served a somewhat similar reaction at 5; but at 4, 6, 7, 8 and 9, the shortening of a time of maximal Straub sign coincides obviously with the pressor episodes.

Graph 2 presents our findings in a second series of experiments, where we have used two groups of mice, using ten animals from each group daily for forty-seven consecutive days. In the first group, the animals were given water with 2 per cent of sodium citrate which raised the pH of their urine up to 8.0 (normally, with our diet, it was around 6.0), and in the second group, the animals received water with  $\frac{1}{30}$  per cent of hydrochloric acid which lowered the pH of their urine to 5.5. The upper black line of Graph 2 presents the findings in

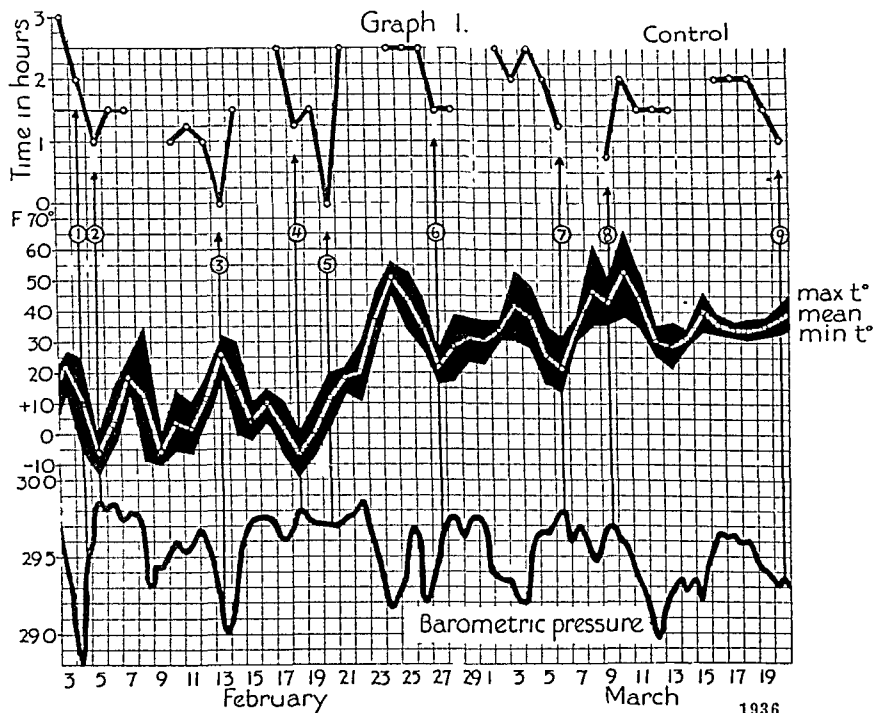
deciduous teeth of the dogs, but marked changes occurred in the enamel of the permanent teeth. The fluorides were fed to the bitches during lactation, and it is possible that had the feeding occurred during pregnancy the deciduous enamel might have been affected. Since the ingestion of certain fluorides has such detrimental effect upon the enamel of permanent teeth, the question arises whether the fluorides present in water might not affect other tissues or organs or metabolic processes of one kind or another. This problem is receiving study in these laboratories at the present time. It has been found in the case of dogs that oral administration of 0.45 mg to 4.52 mg fluorine as sodium fluoride per kilo of body weight caused no effect on total calcium, acid soluble inorganic phosphorus, hemoglobin, or coagulation time of blood. Radiographs showed no changes in the bones of dogs which received as much as 4.52 mg of fluorine as sodium fluoride orally per kilo of body weight.

Experiments have been made to determine the effects of organic fluorides on the enamel of the teeth in the hope that there might be some relation between structure of the compound and the capacity of the organism to split off the fluorine atom. Rats were employed as the experimental animal and the following compounds were tested:  $\alpha$ -fluoronaphthalene, *p*-fluorobenzoic acid, *p*, *p'*-difluorodiphenyl, and fluorobenzene.  $\alpha$ -Fluoronaphthalene was fed at levels of 0.10 and 0.05 per cent of fluorine. Para-fluorobenzoic acid and *p*, *p'*-difluorodiphenyl were administered to the extent of 0.05 per cent of fluorine whereas, fluorobenzene was given at a level of 0.10 per cent of fluorine. The animals receiving 0.10 per cent of fluorine as  $\alpha$ -fluoronaphthalene died in twenty days and showed no abnormalities of the teeth. The same compound fed at a level of 0.05 per cent of fluorine produced mottled enamel. The animals on this latter level of  $\alpha$ -fluoronaphthalene did not reproduce, although they were more than a year old when the experiment was discontinued. The *p*-fluorobenzoic acid, *p*, *p'*-difluorodiphenyl, and fluorobenzene did not produce mottled teeth at the levels used. None of the animals on the organic fluorides showed any significant variation in hemoglobin content of the blood.

Experiments were made on the removal of the fluorine from water. In order to study this problem of removal on a large scale, an experimental plant was attached to the mains in one of the schools in the city of Ankeny. This water treatment machine was designed by Professor W. E. Galligan of the Civil Engineering Department and used by him for water purification, softening, and clarification. The plant consists of three mixing tanks, a settling basin, and filters. Sand and gravel, such as are ordinarily used in the filtering of water supplies, were employed for the filter beds. Analyses of fluorine were made by the Boruff and Abbott<sup>9</sup> modification of the Willard and Winter method. Shortly after the discovery of fluorine in the water at Ankeny, experiments were begun by two of the authors (Greenwood and Nelson) to remove fluorine by the addition of aluminum salts, because of the great insolubility of aluminum fluoride. This work was in progress only a short time when Boruff<sup>10</sup> announced that fluorine may be removed from water by treatment with alum (aluminum sulphate). In the experiments to be described, aluminum sulphate has been used to carry down the fluorine from the water.

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Graph 1.—The upper broken line designates the positive Straub sign in nine or ten mice obtained on the given date. On the left is shown time (in hours) of the duration of the Straub sign from the moment of morphine injection. The wide, uneven, black line expresses the official temperature (maximum, mean, and minimum) on given days. The lower curve is a barograph, showing the official barometric pressure for the days on which the experiments were performed. The figures below give dates on which experiments were done.

served a somewhat similar reaction at 5; but at 4, 6, 7, 8 and 9, the shortening of a time of maximal Straub sign coincides obviously with the pressor episodes.

Graph 2 presents our findings in a second series of experiments, where we have used two groups of mice, using ten animals from each group daily for forty-seven consecutive days. In the first group, the animals were given water with 2 per cent of sodium citrate which raised the pH of their urine up to 8.0 (normally, with our diet, it was around 6.0), and in the second group, the animals received water with  $\frac{1}{30}$  per cent of hydrochloric acid which lowered the pH of their urine to 5.5. The upper black line of Graph 2 presents the findings in

showed very slight mottling, whereas, the females, on these levels during lactation, showed somewhat more extensive mottling. All of the alum fed rats grew normally, and they all reproduced. Second generation animals on the alum diets are now on experiment. Although  $\text{Al}_2\text{F}_6$  did not produce mottled enamel,  $\text{CaSiF}_6$  and  $\text{CuF}_2$  were positive in their effect. The experiments show that alum administration is effective in markedly reducing the effect of fluorine, and, no doubt, with larger quantities of aluminum salts, the effect of fluorine upon the teeth can be prevented entirely.

#### SUMMARY

1 Oral administration of 0.45 mg to 4.52 mg of fluorine as sodium fluoride per kilo of body weight caused no effect on total calcium, acid soluble inorganic phosphorus, hemoglobin, or coagulation time of blood of the dog.

2 Radiographs indicate that there are no changes in the bones of dogs which received 4.52 mg of fluorine as sodium fluoride orally per kilo of body weight.

3 Alpha fluoronaphthylene produced mottled enamel, whereas, the ingestion of *p*, *p'* difluorodiphenyl, *p* fluorobenzoic acid, and fluorobenzene had no effect on the teeth.

4 Fluorine in water has been lowered by treatment to 1.5 to 2 parts per million from an original concentration of 8.0 parts per million.

5 Acidity of the water is a factor in the removal of the fluorine by aluminum sulphate. This is in part due to the nature of the floc produced under different hydrogen ion concentrations.

6 Calcium silicofluoride and cupric fluoride caused mottled enamel, whereas, ingestion of  $\text{Al}_2\text{F}_6$  did not produce this effect.

7 The ingestion of aluminum sulphate, simultaneously along with fluorides, prevents, or at least markedly reduces, the effect of fluorine on the teeth.

#### REFERENCES

- 1 Smith, M. C., Lantz, E. M., and Smith, H. V. Cause of Mottled Enamel, a Defect of Human Teeth. *Ariz Agr Exper Sta Bull* 32: 203, 1928.
- 2 Churchill, H. V. Occurrence of Fluorides in Some Waters of the United States, *Ind & Eng Chem* 23: 996, 1931.
- 3 McKay, F. S. Mottled Enamel: The Prevention of Its Further Production Through a Change of Water Supply at Oakley, Ida. *J Am Dent A* 20: 1137, 1933.
- 4 Kehr, R. W. Dental Deficiencies and Drinking Water, *J Am Water Works A* 28: 214, 1931.
- 5 Ostrem, C. T., Nelson, V. E., Greenwood, D. A., and Wilhelm, H. A. The Occurrence of Mottled Enamel in Iowa, *Science* 76: 575, 1932.
- 6 Boissevain, C. H. The Occurrence of Fluorine in the Drinking Water in Colorado, *J Colorado Wyoming Acad Sc* 1: 13, 1933.
- 7 Dean, H. T. Distribution of Mottled Enamel in the United States, *Pub Health Report* 48: 703, 1933.
- 8 Sebrell, W. H., Dean, H. T., Elvove, E., and Breaux, R. P. Changes in the Teeth of White Rats Given Water From a Mottled Area Compared With Those Produced by Water Containing Sodium Fluoride. *Pub Health Report* 48: 437, 1933.
- 9 Boruff, C. S., and Abbott, G. B. Determination of Fluorides in Illinois Waters, *Analytical Ed Ind and Eng Chem* 5: 236, 1933.
- 10 Boruff, C. S. Removal of Fluorides From Drinking Waters, *Ind & Eng Chem* 36: 69, 1934.
- 11 Keil, H. H., and Nelson, V. E. Aluminum in Nutrition, *Iowa Acad Sc* 41: 161, 1934.

## CONCLUSIONS

1. The duration of the Straub sign in mice is definitely connected with pressor episodes (polar fronts).
2. The mice on alkaline water give a Straub sign of greater duration in comparison with the animals on acid water.
3. The more changes in meteorologic conditions are pronounced, the greater is the duration of Straub sign in both groups of mice, on alkaline or acid diet.
4. The minimal lethal dose of morphine obviously increases the total death rate of all three groups of mice (on ordinary, on alkaline and on acid diet) on the days of pressor episodes.
5. Ordinary, alkaline or acid water in mice diets definitely changes their reaction toward injected minimal lethal dose of morphine sulphate.

I wish to express my sincere gratitude to Dr. Bernard Fantus for his many valuable suggestions.

## REFERENCES

1. Nedzel, A. J.: Variations in the Toxicity of Morphine Sulphate, *J. LAB. & CLIN. MED.* 22: 1031, 1937.
2. Petersen, Wm. F.: The Patient and the Weather, Vol. I-IV, Ann Arbor, Michigan, 1934-36, Edwards Brothers.
3. Herrmann, O.: Biologische Nachweise des Morphins, *Biochem. Ztschr.* 39: 216, 1912.
4. Macht, David J.: Discussion of Fenn's, S. K., and Gilbert's, N. C. paper: Anginal Pain as a Result of Administration of Digitalis, *J. A. M. A.* 98: 103, 1932.

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## INFLUENCE OF DIET UPON THE ACTION OF PHENOBARBITAL SODIUM\*

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PREVIOUSLY we have reported (Nedzel<sup>1, 2</sup>) our observations on reaction of rabbits to cocaine and ethylhydrocupreine hydrochloric injections, the animals being on carrots, mixed, and oats and water diets. It was found that the rabbits on carrot or mixed diet reacted to the injection of cocaine more or less similarly; though the animals on carrot diet reacted more vigorously. The animals on oats and water diet were definitely more sensitive to cocaine poisoning, namely, the toxic effects were greater and recovery was considerably delayed.

In experiments with ethylhydrocupreine injections animals on an oat diet reacted similarly, as in cocaine poisoning experiments; they appeared to be more sensitive to the ethylhydrocupreine than the animals on a mixed diet. But here, contrary to experiments on cocaine poisoning, the rabbits on carrot diet reacted less severely than the animals on mixed diet.

In continuation of studies described, we have undertaken another series of experiments, presented here, replacing cocaine and ethylhydrocupreine hydrochloric with phenobarbital sodium.

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## MATERIAL AND METHODS

Fifty subjects over fifty-five years of age and free from acute infections, diabetes mellitus, and glomerular (hemorrhagic) nephritis, were selected from the wards of the hospital. Because of the age group investigated, the patients were admitted to the institution for a variety of conditions, particularly, heart disease, osteoarthritis, hypertension, and arteriosclerosis. These subjects had all been on the same ward diet for at least a week. The fifty subjects were divided into two equal groups. One group was investigated by the standard method and the other group by the Exton-Rose procedure. The blood sugar was determined by the method of Folin and Wu<sup>7</sup> and the urine sugar by Benedict's method.<sup>8</sup>

## RESULTS

A summary of the results of the fifty cases studied is given in the protocol; the individual blood sugar curves have been omitted in order to conserve space.

The results with the standard test indicate that in the aged the fasting blood sugar is normal, the highest being 113.4 mg. per cent; the peak of the curve is generally delayed varying from one to two hours; the duration of the curve is prolonged, in most instances exceeding two hours. Sixteen cases with high prolonged curves, five with prolonged and four with normal blood sugar curves were observed. Urine volumes were small and in many instances, none could be voided throughout the length of the test. In the second series studied with the one-hour two-dose dextrose tolerance test, the fasting blood sugar levels are comparable to those of the first group. An analysis of these data according to the criteria postulated by Exton and Rose revealed twenty-one diabetic and four normal types of sugar curves.

## COMMENT

Carbohydrate metabolism as measured by blood sugar curves is definitely impaired in old age. Marshall<sup>4</sup> found dextrose tolerance to be diminished more frequently in the old age group with disease than in the normal old age group. In the present series no difference could be found in the blood sugar curves in subjects with heart disease, carcinoma, and arthritis that could not be adduced to old age alone. The blood sugar curves obtained are comparable to those of the twenty-eight healthy subjects of Marshall's series.

TABLE I  
SUMMARY OF TWENTY-FIVE BLOOD SUGAR CURVES OF OLD PEOPLE BY  
THE STANDARD METHOD

	BLOOD SUGAR IN MG. PER 100 C.C.			
	FASTING	AFTER GLUCOSE		
		ONE-HALF HOUR	ONE HOUR	TWO HOURS
Lowest	73.2	118.0	129.2	69.1
Highest	113.4	192.0	227.3	211.3
Average	88.4	146.2	167.5	146.6

severity of the mottling is related to the amounts of each water consumed. Fig. 1 shows typical mottling of the teeth of a child from this region. Fig. 2 illustrates a section of a mottled human tooth.

Rats fed certain fluorides, such as NaF or fluoride water, in all cases developed mottled enamel very similar to that observed in man. Experiments



Fig. 1.—Showing the mottled condition of the permanent teeth of a child.

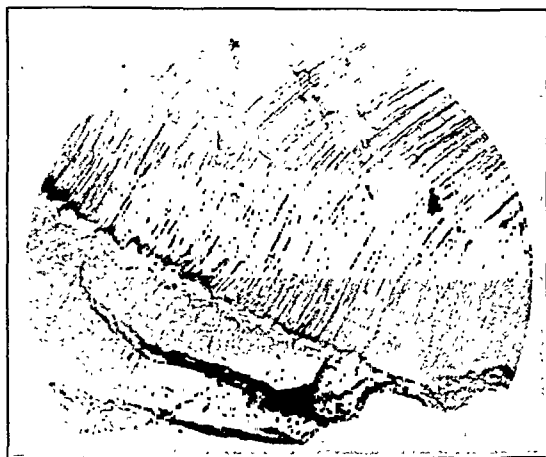


Fig. 2.—Showing section of human mottled tooth.

were made on dogs also, in order to ascertain whether fluorides or fluoride water would produce like results and whether or not the deciduous teeth would be affected. The dog differs from the rat in that it has both deciduous and permanent teeth; whereas, the rat has one set of teeth which, because of a persistent pulp, continue to grow during the life of the animal. The results of these experiments demonstrated that no changes occurred in the enamel of the

## REFERENCES

- 1 Spence, J C Some Observations on Sugar Tolerance With Special Reference to Variations Found at Different Ages Quart J Med 14 314, 1920
- 2 Hale White, R, and Payne, W W The Dextrose Tolerance Curve in Health, Quart J Med 19 393, 1926
- 3 Punschel, A Der Blutzucker im höheren Lebensalter unter der Berücksichtigung der alimentären Hyperglykämie, Ztschr f klin Med 96 253, 1923
- 4 Marshall, F W The Sugar Content of the Blood in Elderly People, Quart J Med 25 257, 1931
- 5 Mosenthal, H O The Interpretation of Sugar Tolerance Tests The Common Occurrence of Renal Glycosuria Med Clin N A 9 549, 1925
- 6 Exton, W G, and Rose, A R The One Hour Two Dose Dextrose Tolerance Test, Am J Clin Path 4 381, 1934
- 7 Folin, O, and Wu H A System of Blood Analysis Supplement 1 A Simplified and Improved Method of Determination of Sugar, J Biol Chem 41 367, 1920
- 8 Benedict, S R The Detection and Estimation of Glucose in the Urine, J A M A 57 1193, 1911
- 9 Nissen, H A, and Spencer, K A Sugar Tolerance in Arthritis, New Eng J Med 210 13, 1934
- 10 Bruger, M, and Mirsky, I A The Variations of the Urea, Total Non Protein Nitrogen, and Chloride Concentration in the Blood Following Glucose Ingestion, J Lab & Clin Med 19 474, 1934

## A STUDY OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ALBINO RAT\*

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**B**RYCE and Burnet<sup>1</sup> have shown that a fair proportion of the domestic rats, mixed black and black and white strains, show staphylococcal antitoxin in their serum. They conclude from their study that the natural antitoxic immunity of rats is acquired as the result of antigenic stimuli from the environment and is not an inborn characteristic. In support of this idea, Bryce and Burnet state that rats possessing natural antitoxin respond to a single injection of toxin with a sharp secondary type response while those lacking antitoxin show no antitoxic response within ten days. Further, with repeated injections of toxin, non immune rats develop antitoxin and very young rats born of natural immune mothers possess antitoxin which later disappears.

This report deals with the active production of staphylococcus immunity, its rate of development and duration, and a study of passive immunity in the young albino rat.

### METHODS AND MATERIALS

The animals used were albino rats of the Wistar strain. Adults were at least 75 days old and weighed 160-275 grams. The young rats were approximately 30 days old when tested for the presence of immunity.

The toxin<sup>†</sup> was prepared by the method of Parker, Hopkins and Gunther<sup>2</sup> with a few unessential modifications.

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†This toxin was prepared by Dr A L Joyner in the Department of Bacteriology, Duke University School of Medicine.

The data in Table I show that the fluorine content of Ankeny city water has been lowered to an appreciable extent. While addition of HCl to the alkaline Ankeny city water improved somewhat the removal of fluorine in small scale laboratory experiments, such improvement was not so marked in the use of the continuous treatment plant at Ankeny. The addition of small amounts of

TABLE I  
THE EFFECT OF ADDITION OF ALUM ON FLUORINE CONTENT OF WATER\*

EXPERIMENT	APPEARANCE OF EFFLUENT	C.C. CONC. HCl ADDED PER GAL. WATER	FLUORINE IN EFFLUENT	
			AS RECEIVED	AFTER STANDING
1	Slightly cloudy	0.16	2.73	---
2	Clear	0.16	2.26	---
3	Clear	0.08	2.02	---
4	Very cloudy	None	3.68	1.88
5	Very cloudy	None	3.00	1.50

\*The dosage of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$  was constant at 20 grains per gallon. The water under treatment contained 8 parts per million of fluorine.

HCl in the continuous treatment caused the flock to settle more rapidly and completely, so that the water from the filter was reasonably clear and, upon standing, gave little sediment. Such was not the case, however, when HCl was omitted, for the flock settled less rapidly and not as completely, resulting in an effluent which was quite cloudy, and which, upon standing, gave much sediment. The cloudy effluents, as received, gave higher values for fluorine than did the clearer ones, but if allowed to stand and then analyzed, sediment free, the originally cloudy effluents gave values lower than the originally clear effluents. We are making more detailed studies of the factors which might influence removal, with the hope of establishing the optimum combination of conditions for practical and more nearly complete removal of fluorine. Laboratory experiments show that alum treatment of solutions of fluorides lowers the fluorine concentration to as much as one part of fluorine per million of solution. When the conditions are known it should, therefore, be possible to remove fluorine from large quantities of water, to a very low level.

Previous work from this laboratory by Keil and Nelson<sup>21</sup> on aluminum in nutrition, indicates that animals can tolerate considerable quantities of aluminum salts. The toxicity of aluminum salts depends on the nature of the salt. Whereas, NaF or  $\text{CaF}_2$ , when fed to rats, causes mottled enamel, it was observed that the administration of 0.10 per cent of fluorine as  $\text{Al}_2\text{F}_6$ , aluminum fluoride, had no effect upon the teeth. The animals looked well and reproduction was very good on this level of aluminum fluoride intake. It, therefore, seemed possible that, if aluminum sulphate were fed to animals receiving fluorine water, mottled enamel would not develop, and no serious disturbance of metabolism should result. Animals receiving 0.025 per cent of NaF all showed severely mottled teeth. The animals which received 0.025 per cent of NaF plus 0.132 per cent of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$  showed some mottling. The male animals receiving 0.025 per cent of NaF plus 0.396 per cent of alum had normal teeth, whereas, the female rats on these levels showed normal teeth until after birth of young, when the mothers exhibited slight mottling. Male animals receiving 0.05 per cent of NaF plus 0.792 per cent of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$

that survived the lethal dose of toxin at frequent intervals during and after the period of immunization. The results of this experiment are given in Chart 1.

DURATION OF THE IMMUNITY TO STAPHYLOCOCCUS TOXIN DEVELOPED  
BY THE ADULT RAT

The duration of the immunity to staphylococcus toxin was determined by immunizing a group of 9 rats with the toxin and giving them the lethal dose ninety-nine days later. All the rats survived. Another group of 5 rats immunized the same as the above was given the lethal dose of toxin one hundred thirty-nine days after completing the immunization. One rat died. The control group of 32 rats was given the lethal dose of toxin and 31 died.

PASSIVE IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE RAT

Female adult rats were given small injections of staphylococcus toxin before, during, and after gestation. Their young were given the lethal dose of toxin at approximately thirty days of age to determine whether or not they were completely protected at this time. In certain cases the mothers were given the lethal dose of toxin shortly after their young were weaned to determine whether or not they were completely immune.

*Experiment I.*—A group of 5 adult females was given 8 injections of staphylococcus toxin for immunization before they were bred.

Table I gives the number of rats born in each of these litters, the average weight and age and the number surviving the lethal dose of toxin.

*Experiment II.*—Four adult females were given injections of toxin at the same time as those in Experiment I. An additional injection of 0.5 c.c. of toxin was also given to these rats two to fourteen days before their litters were cast. Table II shows the result of the lethal dose of toxin on the young rats in this group.

TABLE I

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, the effects of this dose of toxin, and the length of time between immunization and the time the young were born. The mothers were given 8 injections of toxin before they were bred. In the control group of 28 rats, thirty to forty days of age with an average weight of 73 gm, 24 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	DAYS FROM IMMUNIZATION UNTIL LITTER CAST	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM	RESULTS	
					SURVIVED	DIED
6	120	6	28	61	1	5
7	117	6	28	63	1	5
8	120	4	28	55	0	4
9	159	3	28	53	0	3
10	99	6	27	64	1	5
6	152	6	25	37	0	6
7	155	6	27	51	0	6
10	152	6	28	52	0	6

Three more adult females were given 8 injections of toxin before they were bred. A small injection of toxin was also given two to fourteen days before they cast their litters. There were 19 rats in the three litters. Two survived the lethal dose of toxin. In the control group of 13 rats, thirty days of age and averaging 50 gm, 13 died after receiving the lethal dose of toxin.

## DEXTROSE TOLERANCE IN THE AGED\*

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LITTLE attention has been paid to the dextrose tolerance in the aged. Spence<sup>1</sup> was one of the first to show that dextrose tolerance diminishes with advancing years. Blood sugar curves of five men over sixty years of age showed a markedly impaired dextrose tolerance with an average fasting blood sugar of 135 mg. per cent. Hale-White and Payne<sup>2</sup> found in fourteen old people that at the age of seventy the average peak of the blood sugar curve was 220 mg. per cent and at eighty 240 mg. per cent. Punschel,<sup>3</sup> on the other hand, found the average fasting blood sugar in elderly people to be within normal limits. Marshall<sup>4</sup> studied fifty subjects and found that in old people the blood sugar curves fell arbitrarily into five groups: (a) the normal adult, (b) the storage defect, (c) the lag, (d) the flat, and (e) the typical diabetic. The storage type was found to occur most frequently.

### EXPERIMENTAL

Two tests for dextrose tolerance have been employed in the present study. The first, designated as the standard test, is the one in general use and consequently has the most data available for comparative study. In this procedure 100 gm. of dextrose are given by mouth after an overnight fast. Blood and urine specimens are collected prior to, and thirty minutes, one hour, and two hours after, the ingestion of the sugar. The blood sugar curves obtained by this method were analyzed according to the classification proposed by Mosenthal.<sup>5</sup>

The second test of dextrose tolerance used was the one-hour two-dose method advanced by Exton and Rose.<sup>6</sup> This procedure is based chiefly on the fact that normal human beings react to repeated doses of dextrose with either hypoglycemia or with little or no change in glycemia, whereas diabetics react with a definite hyperglycemia. One hundred grams of dextrose are divided into two equal parts. Following an overnight fast, blood and urine specimens are obtained. The first dose of dextrose is then given and thirty minutes later a second sample of blood is obtained. The second dose of dextrose is then given and thirty minutes after this blood and urine samples are again obtained. According to Exton and Rose, diminished dextrose tolerance is assumed if the blood sugar in sixty minutes exceeds that of the thirty-minute specimen by more than 10 mg. per 100 c.c. of blood. The fasting blood sugar and the degree of glycosuria are indicative of diabetes with this as in other tests.

\*From the Department of Medicine, New York Post-Graduate Medical School and Hospital. Received for publication, January 2, 1937

Aided by a grant from the Harriet Weil Fund.

complete immunity to four lethal doses of toxin by the twenty fourth day after the first injection. Immunity apparently begins to decline on the thirty first day after the first injection of toxin as shown in Chart 1.

The duration of complete immunity to the lethal dose of toxin is shown by the survival of rats on the ninety ninth day following the period of immunization. On the one hundred thirty ninth day following the course of immunization some of the rats succumb to the lethal dose of toxin. The number of rats is small, however, the data are interesting in that they are indicative of the approximate duration of immunity in the adult rat.

Passive immunity is demonstrated by the fact that every rat born from immune mothers in Experiments 6 and 7 survived the lethal dose of toxin. The mother in Experiment 6 received a single injection of toxin on approximately the fifteenth day of gestation and four injections of toxin during lactation. The mothers in Experiment 7 received only 4 injections of toxin during the period of gestation. All the mothers in Experiments 6 and 7 survived the lethal dose of toxin. From these two experiments it would seem that the antibodies pass through the placenta, colostrum and milk. Needham<sup>3</sup> states that the rat has a hemochorial type of placenta. Kuttner and Ratner<sup>4</sup> state that antibodies do not pass through the colostrum in animals with this type of placenta. In view of this fact it would seem that some rats respond to a single injection of toxin, given during the period of gestation, sufficiently to cause their young to be completely protected against the lethal dose of toxin at thirty days of age. Experiment 4 shows that a majority of rats however do not develop a sufficient degree of immunity, from a single injection of toxin, to protect themselves or their young from the lethal dose of toxin.

Experiment 5 supports the observations of others that antibodies do not pass through the colostrum in animals with a hemochorial type of placenta. The two mothers in this experiment survived and each of the 16 young rats died following the injection of the lethal dose of toxin.

It seems that rats immunized to staphylococcus toxin and given a small injection of toxin during the latter half of gestation have a higher percentage of immune animals in their litter than the rats given the same number of injections of toxin and fail to receive an additional injection during gestation. This is shown in Experiments 1 and 2.

From this study on passive immunity in the rat it appears that the antibodies reach the young by passing through the placenta. One injection of toxin during the period of gestation is usually insufficient to produce complete immunity in the young and is also insufficient to protect the mother against the lethal dose of toxin. The greatest degree of protection for the young is obtained by giving several injections of toxin during the period of gestation.

The strength of the toxin used for immunizing the rat is important in studying the speed of development and the degree of immunity at different intervals. If the toxin is weak the degree of protection is less than that found in animals given the same amount of a potent toxin after the same interval of time.

TABLE II

SUMMARY OF TWENTY-FIVE BLOOD SUGAR CURVES OF OLD PEOPLE BY  
THE EXTON-ROSE METHOD

	BLOOD SUGAR IN MG. PER 100 C.C.			
	FASTING	AFTER GLUCOSE		
		ONE-HALF HOUR	ONE HOUR	
Lowest	71.8	91.5	105.6	
Highest	106.3	168.5	223.7	
Average	92.9	132.1	173.3	

It need not be assumed that because a large number of the blood sugar curves in the aged showed a diminished dextrose tolerance, diabetes or a tendency to diabetes is present. Mosenthal<sup>5</sup> has shown that there are many so-called normal persons who have blood sugar curves that indicate impaired dextrose tolerance. The work of Nissen and Spencer<sup>9</sup> is of interest in this regard. They studied the dextrose tolerance in 222 cases of arthritis. Fifty-seven per cent showed diminished tolerance and 43 per cent were normal. Most of the subjects under thirty years of age fell in the normal group while most of the older age group showed impaired dextrose tolerance. Serial tests in a group of thirty-three subjects covering periods from one to nine years showed that even a markedly diminished sugar tolerance does not per se indicate a future diabetic. They also found that the diminished sugar tolerance of the arthritic does not always return to normal at the cessation of activity.

The factors responsible for the diminished dextrose tolerance in the aged are not clear. The answer must be sought for in the functional disturbances of various organs and processes in the body. More detailed knowledge about senescence is needed.

The normal youth, when given an excess of glucose, usually responds with a diuresis. None of the elderly subjects showed a diuretic response following the ingestion of dextrose; in many instances there was even difficulty in obtaining specimens of urine. This confirms the observation made by Bruger and Mirsky<sup>10</sup> that diuresis following glucose ingestion occurred more frequently and was more marked in subjects with normal dextrose tolerance than in those exhibiting a diminished tolerance for sugar.

#### CONCLUSIONS

1. Dextrose tolerance tests were carried out in 50 subjects over fifty-five years of age. The results indicate that dextrose tolerance in the aged is impaired although the fasting blood sugar is usually normal.

2. The results obtained with the standard test using 100 gm. of dextrose are comparable to those carried out by the procedure of Exton and Rose.

3. Evidence is added to the fact that while every case of diabetes mellitus exhibits a diminished dextrose tolerance, every case showing diminished tolerance to sugar is not one of diabetes.

# GUANIDINE LIKE SUBSTANCES IN BLOOD\*

## I COLORIMETRIC ESTIMATION AND NORMAL VALUES

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ALTHOUGH a number of methods for the determination of guanidine like substances in blood had previously been described, the first procedure in any sense satisfactory was that outlined by Major and Weber<sup>1</sup> in 1927. This method consists briefly of the following steps. The guanidine like substances in blood are extracted from the Folin Wu filtrate by means of blood charcoal (in a basic solution), and the guanidines released from the charcoal by treatment with cold acid alcohol. This extract is compared colorimetrically with suitable guanidine standards using a modification of Liegs<sup>2</sup> color reagent to develop the color. Since creatine gives an appreciable color with the reagent, a separate creatine determination is made on the final extract, and a correction subtracted from the final result.

About three years later, Pfiffner and Myers<sup>3</sup> described a modification of this procedure. The most important change introduced was the elimination of the creatine correction, by autoclaving the final extract with HCl (thereby converting the creatine into creatinine). Since our work was completed Zapacosta<sup>4</sup> has described another method which is similar to that of Major and Weber, except for the final color development. As a color reagent he uses alpha naphthol and sodium hypochlorite (instead of the ferricyanide nitropruside mixture), the reaction being specific for the methyl derivatives of guanidine. Saunders<sup>5</sup> in 1932, described an entirely different method which appears to actually separate guanidine and its simple derivatives from other compounds giving the same color reaction. However, this method is of little interest in the study of normal or pathologic blood, as it cannot detect (with any accuracy) guanidine compounds below 2 mg per 100 cc (a concentration rarely reached even in pathologic blood).

### EXPERIMENTAL

At the time this work was started, the only satisfactory methods available were those of Major and Weber and of Pfiffner and Myers. Preliminary experimentation soon indicated that the latter procedure was more satisfactory, due to the elimination of the creatine correction. However, several serious objections were found to this method, the more important being as follows: (1) the length of time required, nearly twenty four hours, (2) the low recovery of added guanidine compounds, about 75 per cent, (3) lack of accuracy in colorimeter readings, and (4) the necessity of preparing the color

\*From the Department of Biochemistry, School of Medicine, Western Reserve University. Received for publication February 3, 1937.

The data presented in this and the following papers were taken from the dissertation submitted by Jerome E. Andres to the Graduate School of Western Reserve University, June 1937, in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

A preliminary report of the work presented in this and the following papers was given before the American Society of Biological Chemists, Philadelphia, April 1937. J. Biol. Chem. 97: proc. cix, 1937.

The organism was a hemolytic *Staphylococcus aureus* obtained from an autopsy on a patient whose clinical history was that of agranulocytic angina. The diagnosis was confirmed at the autopsy.

Immunity was produced by intraperitoneal injections of staphylococcus toxin. The presence of immunity in each instance was determined by intraperitoneal injection of a lethal dose of staphylococcus toxin. The survival of an animal following a lethal dose of toxin appears to be a satisfactory criterion for the determination of complete protection. In subsequent work, however, it would be of interest to determine the degree of resistance in terms of staphylococcus antitoxin units

#### PRODUCTION OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ADULT RAT

A total of 25 adult rats were given small injections of staphylococcus toxin for immunization. A lethal dose of toxin was given on the eighth day follow-

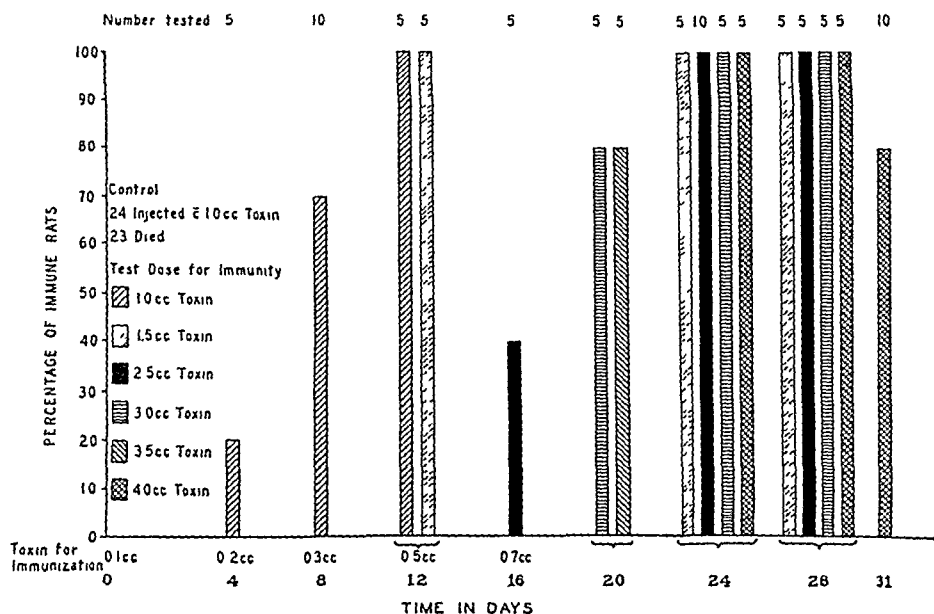


Chart 1—This graph shows the percentage of adult rats surviving the lethal dose of staphylococcus toxin (10 c.c.) at intervals during and after immunization. It also shows the percentage of rats surviving 1.5 c.c., 2.5 c.c., 3.5 c.c., and 4 c.c. of staphylococcus toxin at the different intervals during the experiment. All injections of toxin were given intraperitoneally.

ing the period of immunization. One rat died. A control group of 15 rats was given the lethal dose of toxin and 13 died

A second group of 25 adult rats was immunized in the same manner. No deaths occurred following the injection of the lethal dose of toxin. There were 27 adult rats in the non-immunized group. After receiving the lethal dose of toxin 26 of these rats died within five hours.

#### DEVELOPMENT OF IMMUNITY TO STAPHYLOCOCCUS TOXIN IN THE ADULT RAT

The speed of the development of immunity to staphylococcus toxin was determined by immunizing 95 adult rats and determining the number of rats

used must be large, or the final volume of the extracted material must be quite small. As a rule not over 50 c.c. of blood filtrate are available, and the guanidine compounds from this quantity of filtrate, contained in 4 c.c. of solution (the smallest volume that can be employed with an ordinary colorimeter), give very little color with the nitroprusside reagent. In fact, the color of the 1 c.c. of color reagent added is much greater than that developed by the guanidine. By fitting a colorimeter with micro cups and plungers, it was found that determinations could be easily carried out with 2 c.c. of material. This departure greatly increases the accuracy of the color comparisons with bloods having a low guanidine content. It was also found that as small an amount as 0.12 gm. of charcoal for the extraction of guanidine bases gave better recovery than larger amounts. Smaller amounts of charcoal were not used due to the difficulty in measuring.

Using the ferrieyanide method it was found that guanidine develops about 90 per cent as much color as methyl guanidine. This agrees with Major and Weber's findings,<sup>8</sup> but not with Pfiffner and Myers'.<sup>2</sup> It was also found that creatine gives about one eighth as much color with the color reagent as methyl guanidine, and that the color given by creatinine (within nine minutes) is negligible. This is in accord with both the findings of Major and Weber and Pfiffner and Myers.

TABLE I

THE CONVERSION OF CREATININE INTO GUANIDINES BY THE GUANIDINE METHOD FOR BLOOD

CREATININE ADDED PER 100 C.C. OF BLOOD	DETERMINED AS METHYL GUANIDINE PER 100 C.C.	METHYL GUANIDINE PER MG OF CREATININE
mg	mg	mg
30	0.39	0.0133
5	0.07	0.014
30	0.41	0.0137
20	0.31	0.0155
30	0.46	0.0152
Average		0.014

TABLE II

THE CONVERSION OF CREATINE INTO GUANIDINES BY THE GUANIDINE METHOD FOR BLOOD

CREATINE ADDED PER 100 C.C. OF BLOOD	DETERMINED AS METHYL GUANIDINE PER 100 C.C.	METHYL GUANIDINE PER MG OF CREATINE
mg	mg	mg
20	0.20	0.010
20	0.21	0.0105
30	0.31	0.0103
10	0.09	0.009
20	0.21	0.0105
30	0.30	0.010
20	0.17	0.0085
10	0.09	0.009
Average		0.01

## METHOD

Eight cubic centimeters of oxalated blood are pipetted into a 100 c.c. centrifuge tube, and the proteins precipitated by the Folin Wu procedure, with the exception that the sulphuric acid is added before the sodium tungstate

*Experiment III.*—Two female rats were given four small injections of toxin before they were bred and four injections during the period of gestation. There were 8 rats in the two litters and only 1 survived the lethal dose of toxin.

*Experiment IV.*—Two female rats were given one small injection of staphylococcus toxin on the fifth and eleventh day respectively before their litters were cast. The young rats did not survive the lethal dose of toxin. The two mothers also succumbed to the lethal dose of toxin.

*Experiment V.*—Two females were given three small injections of toxin during the period of lactation. A total of 16 rats were born in the two litters. All died following the lethal dose of toxin. The adult females survived the lethal dose of toxin.

*Experiment VI.*—One female rat was given a single injection of toxin before casting her litter and four injections during the period of lactation.

The four rats in this litter averaged 63 gm. on the twenty-eighth day. At this time they were given the lethal dose of toxin and all survived. The adult rat was given the lethal dose of toxin at this time and she survived. The control group of 50 young rats averaging 57 gm. was given this lethal dose of toxin and 47 died.

*Experiment VII.*—Four females were given four injections of toxin at intervals during the period of gestation. The results obtained from the injection of toxin in both the young and their mothers are given in Table III.

TABLE II

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, and also the effects of this dose of toxin. The females were given 8 injections of toxin before they were bred and one small injection of toxin two to fourteen days before the litters were cast. In the control group of 28 rats, thirty to forty days of age with an average weight of 73 gm., 24 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM.	RESULTS	
				SURVIVED	DIED
1	6	27	63	5	1
2	5	30	70	5	0
3	6	29	59	0	6
4	6	28	61	5	1

TABLE III

This table gives the number of rats in each litter, their age and average weight when the lethal dose of toxin was given, and also the results of this dose of toxin. The mothers were given 4 injections of toxin during the period of gestation and the lethal dose of toxin after the young were weaned. In the control group of 50 rats, twenty-eight to thirty-two days of age with an average weight of 57 gm., 47 succumbed following the lethal dose of toxin.

NUMBER OF MOTHER	NUMBER IN LITTER	AGE IN DAYS	AVERAGE WEIGHT IN GM.	RESULTS		MOTHERS GIVEN THE LETHAL DOSE OF TOXIN RESULTS
				SURVIVED	DIED	
54	6	29	60	6	0	Survived
55	5	30	62	5	0	Survived
56	3	30	68	3	0	Survived
57	3	29	64	3	0	Survived

## DISCUSSION

It is evident from the data that the albino rat is a satisfactory animal to study the immunity produced by staphylococcus toxin. This is shown by the fact that rats developed complete immunity as tested by a lethal dose of toxin given on the twelfth day after immunization was begun. When rats are given five small injections of the toxin at four-day intervals they develop

pared with the appropriate standard in a colorimeter equipped with micro cups and plungers (a Klett biocolorimeter was used). For most bloods the 0.01 standard is sufficient, and unless nitrogen retention is present only the 0.01 and 0.02 mg standard need be prepared. For either the 0.01 and 0.02 mg standards, a graph had best be employed (the graph is prepared by matching the standard against various concentrations of methyl guanidine on either side of the standard and plotting the colorimeter readings of these solutions against their concentration). For the standards containing 0.03 mg or more per 2 cc of volume the guanidine content is proportional to the color, providing the unknown is fairly close to that of the standard.

The reading of the colorimeter should be completed within seven or eight minutes from the time the reagent is added since the color that the reagent gives with creatinine begins to appear in about nine minutes. The guanidine in the sample is calculated (as methyl guanidine) by the following formula, unless the graph is used:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times \text{Concentration of Standard} \times 25 = \text{mg of guanidine per 100 cc of blood}$$

The time necessary to complete a determination is not over three hours.

Since creatine and creatinine are partially converted into guanidine compounds by this procedure (or any other procedure using charcoal in a basic solution), a correction must be applied for this conversion. For creatine this correction amounts to about 0.01 mg of methyl guanidine for each mg of creatine. For the concentration of creatine found in normal blood, this correction amounts to about 0.03 to 0.04 mg of methyl guanidine per 100 cc of whole blood. The correction is quite constant unless nitrogen retention is present.

For creatinine the correction amounts to 0.014 mg of methyl guanidine per mg of creatinine. For the concentration of creatinine found in normal blood this correction amounts to about 0.02 mg of methyl guanidine per 100 cc of blood. This correction is also constant unless nitrogen retention is present.

#### DISCUSSION OF THE METHOD

It is believed that this method presents certain points of improvement over the previous methods for the following reasons:

1 The use of the Folin Wu precipitation reagents in the reverse order insures a more rapid precipitation of the blood proteins the precipitation being quite as complete. The reverse precipitation also gives more filtrate from the same amount of blood. This reverse procedure was also used by Major and Weber.<sup>1</sup>

2 Releasing the guanidine from the charcoal by boiling alcohol has a threefold advantage. It eliminates the guanidine immediately and more completely, and obviates the necessity of allowing it to stand overnight. Second, any charring that might take place on subsequent evaporation, especially the last time, takes place at this time, and the colored material so formed is absorbed by the charcoal. The guanidine compounds are appar-

## SUMMARY

Rats can be immunized to staphylococcus toxin and appear to be satisfactory animals for such a study. Immunity develops at a relatively rapid pace when a small amount of a strong toxin is given at four-day intervals. Rats so treated were completely protected against four lethal doses of toxin on the twenty-eighth day after injections were begun.

A small group of protected rats survived the lethal dose of toxin ninety-nine days after the last immunizing injection.

The antibodies apparently pass through the rat placenta, and not through the colostrum or milk.

Rats, thirty days of age, born from mothers immunized during gestation or parents recently immunized to staphylococcus toxin and given a single small injection of toxin during the period of gestation, have a sufficient number of antibodies acquired through the placenta to produce complete immunity.

## REFERENCES

1. Bryce, L. M., and Burnet, F. M.: Natural Immunity to Staphylococcal Toxin, *J. Path. & Bact.* 35: 183, 1932.
2. Parker, Julia T., Hopkins, J. G., and Gunther, A.: Further Studies on the Production of Staphylococcus Toxin, *Proc. Soc. Exper. Biol. & Med.* 23: 344, 1925-26.
3. Needham, J.: Placental Barrier. *Chemical Embryology*, 1931, 3: Section 21, Cambridge University Press.
4. Kuttner, Ann, and Ratner, Bret: The Importance of Colostrum to the New-Born Infant, *Am. J. Dis. Child.* 25: 413, 1923.

whole blood, with a range of 0.21 to 0.28 mg. The average value for females is 0.23 mg. with a range of 0.18 to 0.28 mg. These values are corrected for the conversion of creatinine into guanidines, but not for the conversion of creatine. When the latter correction is also subtracted, the values are about 0.04 mg. lower.

TABLE IV  
BLOOD GUANIDINE OF NORMAL MALES  
Figures in Mg. per 100 cc. of Blood

NO	AGE	BLOOD UREA NITROGEN	BLOOD SUGAR	BLOOD GUANIDINE
1	39	13	83	0.26
2	27	12	95	0.26
3	27	11		0.26
4	28			0.21
5	23			0.28
6	27	11	103	0.25
7	25	13		0.26
8	30	11	108	0.21
9	23	12	110	0.23
Average				0.25

TABLE V  
BLOOD GUANIDINE OF FEMALES DURING NORMAL PREGNANCY

NO	AGE	BLOOD PRESSURE	BLOOD GUANIDINE IN MG PER 100 CC
1	29	90/50	0.28
2	18	124/80	0.24
3	20	98/50	0.26
4	24	90/58	0.24
5	31	100/50	0.28
6	35	110/70	0.23
7	27	120/70	0.25
8	23	94/58	0.26
9	31	110/70	0.18
10	30	105/72	0.19
11	30	110/60	0.22
12	28	90/50	0.18
13	34	102/60	0.27
14	31	132/96	0.27
15	27	140/82	0.28
16	23	105/77	0.23
17	29	120/85	0.23
Average			0.23

These figures agree favorably with those obtained by other methods. Major and Weber give the normal range for their method as about 0.02 to 0.19 mg. per 100 cc., other workers report values all the way from 0.00 to 0.50 mg. (the figures usually being below 0.30 if the correction for the color given by creatine is subtracted). Pfiffner and Myers' normal figures are between 0.20 and 0.30, others using the method give values for normal individuals ranging from 0.16 to 0.40 mg.

We would like to add that the identity of the substance (or substances) determined by this or similar methods, is not necessarily guanidine or its simple derivatives. Creatinine and creatine have already been mentioned as being adsorbed and released from charcoal in a manner similar to guanidine, and giving some color with the color reagent. Other compounds, notable

reagent previous to each determination. In attempting to improve upon this method, a number of observations were made, the most important of which will be considered briefly.

In the method of Pffner and Myers, the guanidines adsorbed on the blood charcoal are released by allowing the charcoal to stand in contact with acid alcohol overnight. We observed, however, that if the charcoal, containing the adsorbed guanidine, was treated with boiling acid alcohol, better recoveries were obtained than in the overnight treatment with cold alcohol. Furthermore, the liberation of the guanidines seemed to be almost instantaneous. When this modification was introduced into the method of Pffner and Myers, the recoveries of methyl guanidine added to blood amounted to about 82 per cent instead of 75. If the final product was not autoclaved, the recoveries amounted to about 89 per cent.

Further studies showed that treating guanidine and its methylated derivatives with boiling acid alcohol did not destroy any of its compounds. Likewise, creatine and creatinine are apparently not affected by the process.

As previously stated, it was observed that the recovery of methyl guanidine added to blood is about 7 per cent greater when the final product is not autoclaved. In fact, the recovery, without the autoclave, is practically the same as the recovery of pure methyl guanidine in an aqueous solution. Since the autoclaving was shown to have no effect on an acid solution of pure methyl guanidine, the 7 per cent loss in the presence of the blood filtrate is probably due to a chemical combination between guanidine and something in the filtrate, in the presence of the hydrochloric acid.

Notwithstanding this loss, the use of the autoclave to convert creatine into creatinine seemed to be far more desirable than to make a separate creatine determination. The use of creatine correction introduces a variable error much greater than the 7 per cent loss produced by the autoclave. Furthermore, the use of the autoclave definitely shortens the time required to complete a determination and reduces the number of manipulations; in addition, it appears to give more consistent and reliable values.

Major and Weber<sup>1</sup> and also Weber<sup>2</sup> have pointed out the fact that blood charcoal converts creatinine into guanidine bases. Tables I and II show the effect of the blood charcoal method on creatinine and creatine added to blood filtrate. The findings indicate that both creatinine and creatine are converted into guanidine by this procedure, and that for the concentrations found in human blood, the conversion is practically proportional to the concentration of creatine or creatinine present. Part of this transformation is produced by the charcoal in basic solution, and part by the process of autoclaving and evaporation to dryness that follows. While the guanidine substances produced from a given weight of creatine are less than with the same amount of creatinine, the amount of guanidines produced per mol of creatine or creatinine is practically the same. Since Major and Weber did not autoclave their product, their values for the conversion of these two substances into guanidines are lower than the results presented in Table I.

Since the amount of guanidine-like substances in the blood is normally very small, in order to make accurate color comparisons the amount of blood

# SILVER PICRATE TREATMENT OF VAGINAL TRICHOMONIASIS\*

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*TRICHOMONAS VAGINALIS* is believed to be pathogenic in the human vagina and to be the etiologic factor responsible for the symptom complex of vaginal trichomoniasis, or trichomonas vaginitis. It has been definitely established by many workers that control of this infestation in the vagina produces remission of the characteristic symptoms. Two other pathogenic species have been described in lower animals: *T. columbae*<sup>1</sup> in the pigeon and *T. foetus*<sup>2</sup> in cattle. *T. vaginalis* is the type species of the genus *Trichomonas* and is one of the three species which may occur in man (Table I). It has been suggested that these species represent three variants of the same organism, produced by differences in environment, but present evidence does not support this view.

TABLE I  
CHARACTERISTICS OF THE SPECIES OF *TRICHOMONAS* FOUND IN MAN

SPECIES	HABITAT	SIZE (MICRONS)	NO. OF FLAGELLA	NUCLEUS	PARASOL BODY	CHROMATIC GRANULES	UNDULATING MEMBRANE
<i>T. vaginalis</i>	vagina	10 to 36	4	Large, oval, deeply staining	Large, deeply staining	Present	$\frac{1}{4}$ to $\frac{1}{2}$ length of body
<i>T. hominis</i>	intestinal tract	8 to 16	3, 4, or 5	Small, round, less deeply staining	Not demonstrated as yet	Not demonstrated as yet	Entire length of body
<i>T. buccalis</i>	mouth	6 to 12	4	Small, oval, deeply staining	Small, deeply staining	Present	Two thirds length of body

## METHODS OF DIAGNOSIS

The clinical signs associated with trichomonas vaginitis vary, but certain symptoms are more or less pathognomonic of the infestation. There is present in many cases a profuse, creamy, green gray yellow, thin, bubbly and purulent discharge. In addition to the discharge there is often present an eroded cervix, and a vagina showing a profusion of hemorrhagic spots, giving the "strawberry vagina" picture. Burning and itching of the external genitalia is quite common. Dull pain in the lower left and right quadrants is not unusual. Some patients have all these symptoms, others have one or more, while certain individuals harbor the organisms and clinically present no symptoms. The latter are to be regarded as carriers of the disease and should be treated as thoroughly as those showing clinical manifestations.

\*From the Department of Zoology, University of Pennsylvania.  
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The tube is centrifuged and filtered; fifty c.c. of this filtrate are transferred to a 125 c.c. Erlenmeyer flask and rendered basic with 4 or 5 drops of 10 per cent NaOH. To the contents of the flask is added 0.12 to 0.13 gm. ( $\frac{1}{8}$  teaspoon) of blood charcoal (Merek's purified by acid), and the contents shaken well and allowed to stand about two minutes. The contents of the flask are now filtered through a suction filter, using hard filter paper (7 cm. Whatman No. 2). The flask is rinsed with a few cubic centimeters of water containing NaOH (6 c.c. of 10 per cent NaOH per liter), and the liquid poured through the charcoal on the filter paper. The filter paper and charcoal are removed from the filter, and returned to the original flask; 25 c.c. of alcohol, containing HCl (2 c.c. of 10 normal HCl per liter of 95 per cent ethyl alcohol), are added to the flask immediately.

The flask is placed on a water-bath and the contents evaporated to dryness at a temperature between 80° and 90° C. (higher temperatures cause charring). Evaporation is facilitated by drawing a current of air through the flask. To the dry contents of the flask are added 25 c.c. of 95 per cent alcohol, the contents shaken, and allowed to stand a few minutes. The material is then filtered through a hard filter paper (9 cm. Whatman No. 1 is sufficiently retentive), using an ordinary filtering funnel; 20 c.c. of the resultant filtrate are transferred into a 50 c.c. Erlenmeyer flask, and again evaporated to dryness (the temperature of the bath being kept between 80° and 90° C. as before, and the evaporation facilitated by a current of air).

Two cubic centimeters of 0.2 normal HCl are then added to the flask and autoclaved for fifteen or twenty minutes at about 120° C. to convert any creatine present into creatinine. The flask should be covered with an inverted watch glass during the autoclaving. The contents of the flask are again evaporated to dryness as before, the temperature of the water-bath being not much above 80° during the final stages of the evaporation. Then 1 or 2 c.c. of absolute alcohol are added and the alcohol evaporated (this removes the last traces of HCl present).

Two cubic centimeters of water are now measured into the dry flask containing the extract, and standard solutions are made up containing 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, and 0.10 mg. of methyl guanidine† per 2 c.c. of volume (if ordinary cups and plungers are used, the volumes will have to be doubled). To both the standards and unknown is added 0.5 c.c. of the ferricyanide reagent‡ for each 2 c.c. of volume. The unknown is shaken and poured into a 15 c.c. centrifuge tube and centrifuged for two or three minutes to settle the precipitate that always forms at this juncture. The unknown is com-

\*The time factor should be closely adhered to, due to the conversion of creatinine and creatine into guanidine by basic charcoal.

†Methyl guanidine standards are prepared by dissolving 167.1 mg. of methyl guanidine sulphate in 100 c.c. of distilled water. Dilutions of this standard in the ratio of 1:10 and 1:100 are the most convenient for use. The standard keeps perfectly in a refrigerator. Guanidine can be used instead of methyl guanidine, but it develops less color.

‡The reagent is the one described by Major and Weber.<sup>1</sup> It is prepared by mixing the following

- 1 part of 10 per cent sodium hydroxide.
- 1 part of 10 per cent sodium nitroprusside.
- 1 part of 10 per cent potassium ferricyanide
- 9 parts of distilled water (preferably  $\text{NH}_4$  free)

The prepared reagent must stand twenty minutes before using. The reagent will keep long periods of time if kept in a refrigerator in brown bottles. A slight precipitate gradually forms at the bottom ( $\text{Fe}(\text{OH})_3$ ), but does not appreciably affect the reagent.

two hours, depending on the viability of the culture. The addition of 0.01 gm of powdered gastric mucin to each Ringer Loeffler tube is advantageous in maintaining the culture over a long period of time.

#### METHODS OF TREATMENT

In recent years many methods of treatment and many germicidal agents have been used by various workers in the field, with varying degrees of success. A method which has been quite universally employed involves scrubbing the vagina with tincture of green soap, followed by washing with cold water and drying thoroughly. After this procedure the vagina is irrigated with an antiseptic or germicidal solution.<sup>4</sup> However, in cases where the vaginal tract is tender due to an eroded mucosa this scrubbing process is quite irritating. The patient may be relieved during the period of this treatment but it may not be very effective in permanent control of the infestation.

Another type of treatment widely used is the tablet method.<sup>5</sup> In this method too much dependence is placed upon the ability of the patient to insert a tablet as far back as the region of the cervix and fornices. This method does not permit efficient dispersion throughout the entire vaginal tract and the medication may not penetrate all rugae which harbor the organisms.

A preferable form of treatment is the procedure of blowing powder into the vagina while the vaginal tract is distended.<sup>6</sup> In order to accomplish this an instrument must be used which will occlude the entrance to the vagina and permit moderate distention with air by means of a hand bulb. The dispersion of the powder and the distention of the vagina occur simultaneously in the instrument we have used. There is no danger of blowing powder into the fallopian tubes because the pressure can be regulated at the bulb. This enables the medication to penetrate all of the crevices and the rugae and to extend to all parts of the vaginal tract, covering the entire vaginal mucosa and external genitalia.

Among other compounds, picric acid and silver nitrate have both been used in the treatment of *T. vaginalis* vaginitis, but the corrosive action of the latter has been objectionable. Silver picrate has been shown to be more rapidly toxic<sup>7</sup> to *T. vaginalis* in vitro than other silver compounds studied and its use in conjunction with kaolin thus combines a definite toxic action with a useful drying effect.

In the present study, silver picrate\* was used in treating 25 cases of *Trichomonas vaginalis* vaginitis. Five grams of a 1 per cent dispersion of silver picrate in kaolin were used, applied to the vagina by means of an occluding insufflator. This was followed by a course of 6 suppositories, each containing 2 gr of silver picrate. One suppository was inserted by the patient every night, starting the day after insufflation. The patient was instructed to return in one week for another insufflation and 6 more suppositories. Two such treatments were the usual routine. However, more than 2 treatments were given in several cases, as described below. The patients were asked to return after each menstrual period for a re-examination, which was repeated for varying periods of time from about three months to nine months.

\*Supplied through the courtesy of John Wyeth & Brother, Inc., Philadelphia.

ently not affected by this process. Finally, this process adds to the accuracy, as a definite amount of liquid can be added to the dry material, giving a known total volume.

3. The use of the suction filter allows rapid filtering and leaves less liquid adhering to the filter paper and charcoal to dilute the acid alcohol added subsequently.

4. The use of a current of air to facilitate evaporation greatly speeds up the method. It is especially important in removing the hydrochloric acid after autoclaving.

5. The use of a microcolorimeter enables the color comparisons to be made with a greater degree of accuracy.

6. The reagent need not be prepared previous to each determination.

7. The use of smaller amounts of charcoal gives a higher recovery of added methyl guanidine, and probably also of the guanidine-like substance in the blood filtrate.

The corrections for creatine and creatinine are practically constant in normal cases, and need not be applied to pathologic bloods except in cases with nitrogen retention. In all the papers of this series the correction for the creatinine conversion was applied, but the correction for the creatine conversion was omitted.

The recovery of methyl guanidine added to blood, as shown in Table III, averages about 82 per cent with this method.

TABLE III

THE RECOVERY OF METHYL GUANIDINE ADDED TO 50 C.C. OF BLOOD FILTRATE

METHYL GUANIDINE ADDED PER 100 C.C. OF BLOOD	METHYL GUANIDINE RECOVERED PER 100 C.C.	PERCENTAGE RECOVERY
mg.	mg.	
2.00	1.65	83
2.00	1.65	83
2.00	1.63	82
2.00	1.65	83
2.00	1.69	85
1.50	1.18	79
1.50	1.17	78
2.00	1.60	80
2.00	1.60	81
2.00	1.66	83
4.00	3.12	78
2.00	1.67	84
2.00	1.68	84
2.00	1.66	83
2.00	1.76	88
2.00	1.61	81
2.00	1.64	82
2.00	1.60	80
1.00	0.78	78

Average = 82%

Tables IV and V give the results of blood guanidine determinations on 9 normal males and 17 normal females (during the early part of a normal pregnancy). The males give an average value of 0.25 mg. per 100 c.c. of

## THE EFFECT ON THE HEART OF EXPERIMENTAL PLEURAL CONGLUTINATION\*

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### INTRODUCTION

THE idea that extensive conglutination of the visceral and parietal pleurae, particularly in the basal and phrenic regions interferes with ventilation of the lungs, and thus leads to hypertrophy of the right ventricle, is very old, but from the beginning there was no unanimity on the subject. Ludwig Traube<sup>1</sup> called attention to a passage in Gerhard van Swieten's *Commentarii in Hermann Boerhaave aphorismos de cognoscendis et curandis morbis* (vol 2, p 762, paragraph 843), in which observations recorded in the seventeenth century by Jan Baptista van Helmont, Johann Conrad Peyer, Theophile Bonet, and Isbrand van Diemerbroeck were invoked sometimes for, and sometimes against, the idea. Whereas in Peyer's case the widespread pleural synechiae discovered post mortem were held responsible for the respiratory embarrassment which had existed during life, "nevertheless it is not to be denied that van Diemerbroeck found the lungs of an executed criminal so firmly adherent not only to the pleura, but also to the entire mediastinum and diaphragm, that they could not be removed without tearing them to pieces. Yet he had enjoyed excellent health, without any respiratory difficulty"†. A century later both Morgagni and Joseph Lieutaud adopted Peyer's point of view, and added the suggestion that obliterative pleurisy might give rise to cardiac hypertrophy.

It was not until the latter part of the nineteenth century, after the contributions of James Carson,<sup>2</sup> David Barry,<sup>3</sup> and Frans Cornelius Donders<sup>4</sup> had led to a much clearer understanding of the favorable influence which normal respiration exerts on the movement of blood into and within the thorax, that the possibility of a causal relationship between pleural conglutination and selective right ventricular hypertrophy was considered seriously. As a matter of fact, such a relationship was implicit in Donders' work, but Baumler<sup>5</sup> and Traube,<sup>1</sup> by correlating ante and post mortem observations, made the first deliberate attempt to substantiate it. Then evidence, however, like that of all case reports on the subject, is conflicting and inconclusive. The first of

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†Neque dissimulandum tamen est quod Diemerbroeckius invenerit in cadavere suspensuribus pulmonem in utroque latere non tantum pleurae sed et toti mediastino et diaphragmati adeo firmiter accretum ut non sine dilaceratione avelli posset. Interim optime sanus vixerat absque ulla respirandi difficultate.

arginine,<sup>9</sup> behave similarly. However, none of these substances (except creatine and creatinine) have been shown to be present in blood in sufficient quantities to exert an appreciable effect on the final color.

#### SUMMARY

1. A modification of Pflüger and Myers' method for the colorimetric determination of guanidine bases in the blood is described. The method as modified gives about 82 per cent recovery of added methyl guanidine, and takes less than three hours to complete.

2. Creatine (as well as creatinine) has been shown to be partly converted into guanidine bases by the use of charcoal. Using the method as described, 1 mg. of creatine is converted into about 0.01 mg. of guanidine bases (determined as methyl guanidine), while 1 mg. of creatinine is converted into 0.014 mg. of guanidine bases.

3. The concentration of guanidine-like substances in the blood of 26 normal individuals was determined. For males the average value was 0.25 mg. and for females 0.23 mg. per 100 c.c. of whole blood.

#### REFERENCES

1. Major, R. H., and Weber, C. J.: Possible Increase of Guanidine in the Blood of Certain Persons With Hypertension, *Arch. Int. Med.* 40: 891, 1927.
2. Tiegs, O. W.: A Color Test for Guanidine Bases Together With Some Physiological Applications, *Australian J. Exper. Biol. M. Sc.* 1: 93, 1924.
3. Pflüger, J. J., and Myers, V. C.: On the Colorimetric Estimation of Guanidine Bases in the Blood, *J. Biol. Chem.* 87: 345, 1930.
4. Zappacosta, M.: A New Method of Determining Methyl Derivatives of Guanidine in the Blood. Preliminary Observations in Essential and Renal Hypertension, *Boll. soc. ital. biol. sper.* 10: 705, 1935.
5. Sakaguchi, S.: Über eine neue Farbenreaktion von Protein und Arginin, *J. Biochem. Tokyo* 5: 25, 1925.
6. Saunders, J. A.: A Method for the Isolation of Guanidine, *Biochem. J.* 26: 801, 1932.
7. Weber, C. J.: The Determination of Guanidine Bases in the Blood, *Proc. Soc. Exper. Biol. & Med.* 24: 712, 1927.
8. Major, R. H., and Weber, C. J.: The Probable Presence of Increased Amounts of Guanidine in Blood of Patients With Arterial Hypertension, *Bull. Johns Hopkins Hosp.* 40: 85, 1927.
9. Zappacosta, M.: L'Arginine come causa di aspecificità dei metodi di dosaggio della guanidinemia, *Diag. e tec. di lab.* 5: 919, 1934.

of fact, there is no point in studying pathologic material except to learn whether right ventricular hypertrophy invariably accompanies pleural conglutination. If it does, a relationship of some kind may be suspected, but anything resembling proof that the one inevitably entails the other is quite impossible. Only the negative viz, that they are not directly related, is susceptible of proof by such means.

Theoretically, it is not unreasonable to suppose that some relationship exists. The early work of Carson, Barry, and Donders (*loc. cit.*) has been confirmed and extended by Hasse,<sup>8</sup> Wenckebach,<sup>9</sup> and Burekhardt,<sup>10</sup> proving beyond question that normal breathing exercises a beneficial effect on blood flow, and Hoffbauer<sup>11</sup> and Romberg<sup>12</sup> have argued very plausibly that adhesion of the pleurae impairs this function to so great an extent that the right ven-



Fig 2—Exper 18B. Photograph taken at necropsy. Arrows point to costal and diaphragmatic adhesions.

tricle must undergo hypertrophy to compensate for the loss. Romberg maintains that the complete obliteration of one pleural sac is sufficient to bring this about.

As far as we have been able to ascertain, our attempt to elucidate the subject by experimental means is the first that has ever been made.

#### METHOD

The method of producing pleural conglutination which we employed has been described in detail elsewhere.<sup>13</sup> Briefly, it consisted in placing cotton gauze between the visceral and costal pleura, and over the pleural surface of the diaphragm. Figs. 1 and 2 illustrate, as well as photographs can, the nature of the conglutination which resulted. The hearts were prepared and sectioned by Herrmann's<sup>14</sup> method, the ventricles weighed separately, and the ratio of

For establishing a definite diagnosis, the patient is placed in the lithotomy position and a sterile, unlubricated speculum is inserted into the vagina. A smear is taken, by means of a sterile wooden applicator, from the regions of the cervix, fornices, external os, and vaginal wall, and the applicator is then placed in a test tube containing three or four c.c. of modified Ringer's solution<sup>3</sup> (NaCl 6.0 gm.; KCl 0.1 gm.; CaCl<sub>2</sub> 0.1 gm.; NaHCO<sub>3</sub> 0.1 gm.; distilled H<sub>2</sub>O 1,000 c.c.). This tube may be kept at room temperature for three or four hours, although it is preferable to make the examination immediately. A few drops of the diluted vaginal specimen are placed on a microslide for examination under the high power dry lens of the microscope. The presence of the organism is readily detected by its motility. This method of diagnosis has proved superior to other procedures and makes possible recognition of varying degrees of infestation. Thus in first grade or mild cases there are few leucocytes, trichomonads, diplococci, or streptococci, while epithelial cells and Döderlein bacilli are numerous. In third grade or severe infestations, this situation is reversed and the field shows few if any epithelial cells or Döderlein bacilli, but there are large numbers of leucocytes, trichomonads, diplococci, and streptococci. Intermediate or second grade cases show varying proportions of the above two main groups of cellular elements. The dark field, hanging drop and glass coverslip methods are not necessary for diagnostic purposes nor is the addition of dyes to stain the surrounding medium in order to make the organisms more distinct. The organism may be seen more distinctly by cutting off some of the light in the microscope.

*Staining Method (Giemsa).*—The preparation of a permanent slide is not necessary for diagnostic purposes but is necessary where a morphologic study is being made. For this purpose the following method may be used:

1. The material is spread on a microslide or a coverslip by means of a curved forceps, in a thin even film so that it will dry uniformly.
2. Before drying can occur, hold over the mouth of a bottle containing 1 per cent or 2 per cent osmic acid for five to ten seconds.
3. Dry as rapidly as possible, without heat, or the moderate heat of an electric bulb.
4. Fix by immersing in pure methyl alcohol for five to fifteen minutes.
5. Remove and dry in air.
6. Prepare stain by diluting stock Giemsa stain 1:20 with neutralized distilled water, or buffer solution with pH of 6.7.
7. Add dilute stain to smear and leave for thirty to forty-five minutes.
8. Wash in tap water for five seconds.
9. Dry thoroughly without heat.
10. Mount in balsam or examine unmounted with high power dry, or oil immersion lens.

*Culture Method.*—Inoculate with 1 c.c. of the diluted specimen 10 c.c. of sterile modified Ringer's solution to which has been added, immediately before the inoculation, 0.01 gm. Loeffler's dehydrated blood serum in powdered form. The latter is allowed to settle to the bottom of the tube before the inoculation. Incubate at 37.5° C. for twenty-four hours. Remove a small quantity of the culture from the bottom of the tube by means of a pipette and examine for motile organisms. If present, subculture into a fresh tube of Ringer-Loeffler medium. Subsequent subcultures should be made every forty-eight to seventy-

the left ventricular weight to the right ventricular weight compared with Herrmann's ratios for normal dogs' hearts. Seventeen experiments were completed successfully. The following (19B) is a typical protocol.

Description Collie, female

First operation, Jan 24, 1934

Weight 10.7 kg

Operators Cooper, Gregory

Procedure Five strips of gauze inserted in the left pleural sac in the usual manner  
Recovery uneventful

Second operation, May 29, 1934

Operators Cooper, Gregory

Procedure Five strips of gauze inserted in the right pleural sac, and the right half of the diaphragm covered with gauze. Recovery uneventful

Necropsy, June 5, 1935

Weight 14.0 kg

Cause of death Opening the thorax under ether anesthesia

Right pleural sac Only the apical area free of adhesion, the remainder, including the entire phrenic surface completely obliterated. A few pleuroparietal adhesions.  
Draining sinus externally

Left pleural sac One third of lateral area conglutinated. Phrenicocostal sinus full of adhesions, diaphragm otherwise free

Ventricular weights (Gregory)

Left ventricle 48.2 gm

Right ventricle 33.1 gm

L/R ratio 1.45

## RESULTS

The experimental data are presented in Table I. Most of the animals showed a tendency to gain weight during the period of observation. In five experiments pleuritic adhesions were produced on both sides, and in all but two (3B and 22B) the diaphragmatic pleura participated in the process. The estimated extent of the conglutination varied from 10 to 65 per cent of the total bilateral pleural area, and the duration ranged from five to twenty-eight months. All of the L/R ratios fell within the limits of normal established by Herrmann except the one marked with an asterisk (13B). This is probably an experimental error, but if it were correct it would indicate hypertrophy of the left ventricle, not the right. There is nothing to suggest that the moderately large empyemas which occurred in two animals (14B and 17B) made any difference in the results.

## COMMENT

It is obvious that an investigation of this kind should include complete, as well as partial, obliteration of both pleural cavities. It is missing simply because no method capable of producing it was available when the work was being done. If experiments now in progress succeed in supplying the deficiency, a supplementary report will be forthcoming.

In interpreting these experiments, it must not be forgotten that the animal on which they were performed is pronograde. What effect the erect posture might have on the results is purely a matter of conjecture.<sup>15</sup>

husbands. In the fourth case (Case 8) symptoms reappeared after the menstrual period following the two weeks' treatment. Three additional treatments were given to this patient and she remained free of symptoms for the six months she was under observation.

#### SUMMARY

1. A method of diagnosis of *Trichomonas vaginalis* vaginitis is given, including procedures for staining and culturing the organism.

2. Silver pierate, in a 1 per cent dispersion on kaolin, was found to be efficient in rapidly controlling the infestation in twenty-five cases.

3. Methods of transmission are discussed including the presence of *T. vaginalis* in prostatic fluid, which was responsible for recurrence of symptoms in three cases.

The authors wish to express their appreciation to Dr. David H. Wenrich for guidance during the course of this study.

#### REFERENCES

1. Bos, A.: Über Trichomoniasis bei Tauben, Zentralbl. f. Bakt. 126: 550, 1932.
2. Abelein, R.: Die Trichomonadenseuche des Rindes und das Scheidenkatarrhproblem, München. tierärztliche Wchnschr. 83: 318, 1932.
3. Drbohlav, J. J.: Une nouvelle preuve de la possibilité de cultiver Endamoeba dysenteriae, type histolytica, Ann. de parasitol. hum. et comp. 3: 349, 1925.
4. DeLee, J. B.: Trichomonas Vaginalis Vaginitis, Illinois M. J. 37: 186, 1920.
5. Karnaky, K. J.: Trichomonas Vaginalis Vaginitis, Urol. & Cutan. Rev. 38: 174, 1934.
6. Gellhorn, G.: Treatment of Trichomonas Vaginitis With Acetarsone (Stovarsol), J. A. M. A. 100: 1765, 1933.
7. Shelanski, H. A.: Studies on T. Vaginalis In Vitro, J. LAB. & CLIN. MED. 21: 790, 1936.
8. Adair, F. L., and Hesseltine, H. C.: Histopathology and Treatment of Vaginitis, Am. J. Obst. & Gynec. 32: 1, 1936.

1829 PINE STREET

# LABORATORY METHODS

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## AN ASPIRATOR FOR REMOVING FLUID AND AIR FROM THE PLEURAL CAVITY\*†

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BURGESS GORDON M.D. PHILADELPHIA, PA

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VARIOUS methods have been suggested for the removal of air and fluid from the pleural cavity. Their value depends essentially upon the use of suction, needles alone are rarely effective.

The simplest procedure is to employ an ordinary glass syringe, which for the removal of small amounts of fluid is entirely satisfactory. With large quantities the syringe must be detached from the needle repeatedly for emptying, which is tedious, inaccurate and not without danger because of possible contamination. The syringe is also used for the aspiration of air but with limited value. It is difficult to measure the amount of air withdrawn, and there is no means to determine the degree of intrapleural pressure without employing a special needle for connecting with the manometer. A further objection is that air will be sucked back into the pleural cavity while the syringe is being emptied, unless the finger is immediately placed against the needle. As with the aspiration of fluid, the technique is awkward and time consuming.

The Samuel Robinson artificial pneumothorax apparatus is sometimes used for the withdrawal of air. For this purpose the position of the bottles which is ordinarily employed for the introduction of air is reversed. Technically, the procedure is satisfactory, but it may be criticized because of the danger that air will contaminate the apparatus with the possibility that the next patient who receives a pneumothorax treatment will become infected.

A Potam aspirator or one of the modifications is an efficient device for the removal of fluid if a marked negative pressure is maintained in the bottle. An objection is that the bottle and connections are difficult to clean and the latter are not easily kept in good working order. Buell's bottle is used chiefly in connection with the introduction of air in patients receiving artificial pneumothorax treatments. Gordon's pneumothorax decompressor is intended only for the continuous removal of air in patients with persistently high intrapleural pressures.

The present apparatus has been designed in order to facilitate the removal of fluid and air from the pleural cavity. It consists of a graduated glass syringe enclosed in a metal casing (*D*), the metal plunger is accurately ground to fit the barrel of the syringe and has a T shaped handle for convenient manipulation. A metal two way check valve with a ground in cone (*B*) opens

\*From the Department for Diseases of the Chest Jefferson Hospital

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†The instrument is manufactured by The George F. Pilling & Son Company Philadelphia Pa.

Bäumler's three cases was that of a man, aged thirty-four years, who died of cardiac failure. The pronounced hypertrophy and dilatation of both ventricles discovered at necropsy were ascribed, for no better reason than the lack of any other explanation, to the fact that both pleural cavities were completely obliterated. The second and third cases were invalidated by the presence of pathologic conditions other than oblitative pleurisy which might have contributed to the cardiac hypertrophy and dilatation.

Traube did not share Bäumler's opinion that the left ventricle, as well as the right, is affected by pleural conglutination, but his attempt to demonstrate that there is any effect at all was no less unconvincing than Bäumler's. In the report of the only one of his three cases in which an autopsy was performed, there was nothing except pure conjecture to indicate that the con-

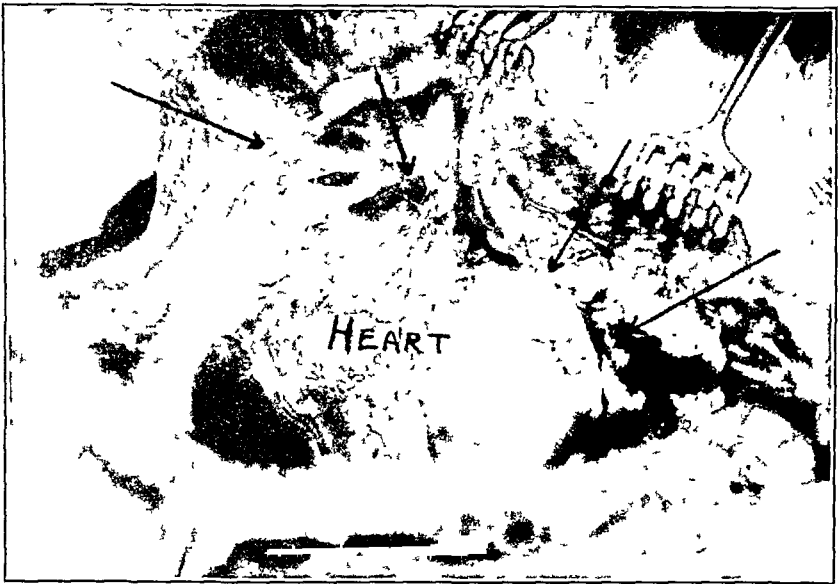


Fig 1.—Exper. 15B. Photograph taken at necropsy. Complete obliteration of right pleural sac indicated by arrows.

junction of complete bilateral pleural conglutination, bronchial catarrh, bronchiectasis, and hypertrophy and dilatation of the right ventricle was anything but fortuitous. The other cases were dismissed summarily with unconfirmed clinical diagnoses. Thus, in spite of the fact that Traube and Bäumler have been cited authoritatively innumerable times, it is quite obvious that their evidence was purely presumptive.

Hirsch's<sup>6</sup> report, based on five cases, is likewise inconclusive. He did not describe his cases in sufficient detail, or take care to protect his thesis against the possible criticism that there may have been other factors in the right ventricular hypertrophy. His laudable endeavor to verify the hypertrophy by employing Müller's<sup>7</sup> method (now known to be inaccurate) of separating the ventricles for weighing did not fortify his argument in the least. As a matter

the handle of the syringe should be pushed forward in order to discharge the contents. This procedure is repeated as many times as necessary, careful count being taken of the amount of fluid withdrawn. In a pneumothorax there is the same manipulation of the syringe but periodically valve "A" should be turned in order to determine the degree of intrapleural pressure. The amount of air withdrawn and discharged will be recorded in cubic centimeters as in the case of the fluid.

In cleaning the instrument at least 1000 c.c. of clean water should be drawn into the syringe and discharged. It is advisable to follow this with 40 c.c. of alcohol and subsequently with 40 c.c. of distilled water.

The advantages of the apparatus are that air and fluid may be conveniently removed and accurately measured; the syringe is fastened to a table and may be manipulated with one hand while the other holds the thoracentesis needle; fluid may be easily drawn through a No. 19 gauge needle in which an ordinary adaptor is used; the intrapleural pressure in pneumothorax cases may be noted at any given time; the apparatus is easily cleaned.

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## A SIMPLE INEXPENSIVE DEVICE FOR THE REGULATION OF GASEOUS PRESSURES\*

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AARON EDWIN MARGULIS M.D. MT. MORRIS, N. Y., AND  
GEORGE BLEZINGER, TUCSON, ARIZ.

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A SIMPLE, easily constructed and inexpensive device is herein described that will maintain the gaseous pressure in a train of apparatus constant at predeterminable and adjustable levels irrespective of fluctuations (within certain limits) of the source of supply (i.e., pump, aspirator, tank, etc.). Such an apparatus has many applications both in the laboratory and in the clinic. Essentially, it consists of a mercury trap with a mercury reservoir so connected that the height of the mercury column in the valve, and therefore the maximum pressure that can occur in the system, may be varied without interrupting operation. By the transfer of a single rubber tubing connection the apparatus may be used for the regulation of either sub- or supra-atmospheric pressures.

The essential features of the instrument are diagramed in Fig. 1. "A" is a heavy-walled Pyrex test tube one inch in diameter. The bulb end was cut off and the rim flanged by heating and pressing against a hard surface. A single-hole rubber stopper was then forced through the tube from the other end, resulting in a non-ejectible closure. A simple two-way stopcock was then inserted into the rubber stopper so that the end of the glass tubing was flush with the upper surface of the stopper. The upper end of "A" was fitted with a two-holed rubber stopper. Through one hole was passed a short piece of glass tubing "S," which just cleared the lower surface of the cork, while through the other was inserted a piece of glass tubing "L," of such length as to extend

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\*From the Laboratories of the Desert Sanatorium of Southern Arizona.  
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TABLE I

EXPER.	BODY WEIGHT (KILOGRAMS)			PLEURAL CONGLUTINATION				L/R RATIO NORMAL: MIN. 1.153 AV. 1.393 MAX. 1.773	REMARKS
	FIRST OPERATION	SECOND OPERATION	POST- MORTEM	LOCATION	EXTENT; IN PER CENT OF TOTAL PLEU- RAL AREA	DURATION (MONTHS)			
						ONE SIDE	TWO SIDES		
1B			13.8	One-half of the diaphragm only	15	28		1.438	
3B	6.8			Costal only	30	16		1.46	
4B	15.7		13.2	One-half of the diaphragm and small costal area	25	27		1.466	
5B	7.5			Entire diaphragm only	40	15		1.712	
8B	7.1			One entire pleural cavity	50	17½		1.223	
10B			9.3	Costal and phrenic	35	26½		1.4	
13B			9.5	Costal and phrenic	40	24		1.94*	
14B			4.7	Costal and phrenic	30	16		1.301	Empyema; one lung partly collapsed. Animal emaci- ated
15B			11.3	One entire pleural cavity	50	24		1.44	Very small encapsulated em- pyema
16B			12.3	Pleuropericardial and phrenic	10	25		1.49	
17B	9.2	• 12.5	10.7	Right: Costal and phrenic. Left: Costal	65	8	4	1.37	Large empyema and collapse of lung
18B	6.5	7.6	9.0	Costal and phrenic, both sides	25	17	12	1.28	Extrathoracic infection
19B	10.7		14.0	Costal and phrenic, both sides	55	16	12	1.45	Extrathoracic infection
20B	7.5	10.5	13.0	Costal and phrenic, both sides	35	16	12	1.4	
21B	9.5	9.0	12.5	Right: Negligible. Left: Costal and phrenic	25	12½	12	1.7	
22B	10.2	11.5	9.0	Costal only	10	5		1.57	Partial atelectasis; small en- capsulated empyema
23B	7.5		7.6	Costal and phrenic	40	5		1.49	
				Average	34				

all three channels are interconnected i.e., the apparatus to be evacuated is cut in. The water pump can then be turned down so that the air bubbles into the chamber "A" in a gentle stream. If it is desired to change the pressure in the system, this can be done without interrupting operation by raising or lowering the level of the mercury in "F" while observing the manometer "F". By turning the stopcock "C" so that only the two lateral arms are interconnected, the valve can be cut out of the system. If it is desired at any time to raise the pressure in the apparatus, gradually to obviate backflow, this may be done by

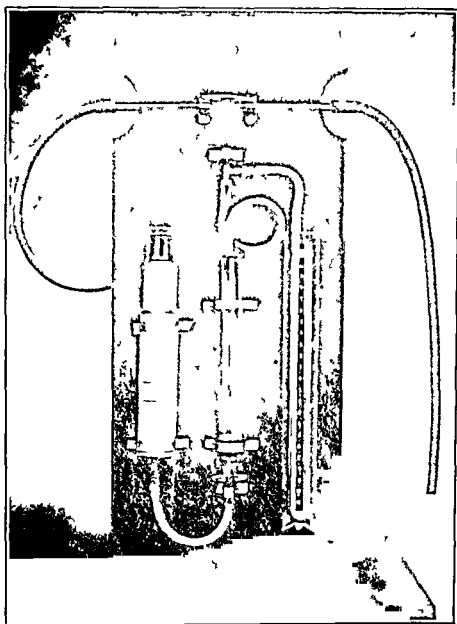


Fig 2

turning the valve "D" so that the mercury valve is connected only with the side connected to the apparatus, and slowly lowering the level of the mercury in the tube "A".

If the apparatus is to be used to control supraatmospheric pressures, the rubber tubing from "D" is connected to "L" instead of "S". In all other respects the operation of the instrument is the same. As an added bit of elegance, a four way glass stopcock, with three side arms and the fourth channel discharging through the cock may be inserted between "D" and the mercury valve "A" to facilitate this interchange.



being allowed to expire through the third arm of the additional three way valve leading to a tube dipped a few millimeters below a mercury column, which controlled the degree of pulmonary deflection desired. Other uses of the above apparatus will suggest themselves.

#### SUMMARY

A simple, easily constructed inexpensive sub and supraatmospheric pressure valve employing the principle of the mercury trap is described which is of use in many laboratory and clinical situations.

### EXAMINATION OF SUSPECTED SEMEN STAINS FOR SPERMATOZOA\*

W W WILLIAMS M D SPRINGFIELD, MASS

**O**CCASIONALLY in legal medicine it becomes desirable to determine whether stains in articles of clothing etc. are due to semen. By using suitable methods such may often be determined by the recovery of spermatozoa from the stains.

It happens that spermatozoa once dried on clothing or elsewhere maintain their morphologic characteristics and staining properties over long periods of time, particularly if the articles in question have been kept dry. Then a permanent stained microscope slide preparation of the spermatozoa may readily be prepared from washings of the soiled cloth. The following is the procedure which I have used in detecting spermatozoa. A small piece of the soiled cloth, usually not more than one half inch diameter, is used. This is placed upon a clean microscope slide, a few drops of saline solution placed upon it, and with the blunt edge of a scalpel the surface of the cloth is scraped off carrying with it any spermatozoa which may be present. The few drops of fluid are spread over the microscope slide. After drying, the film is fixed with heat, and then stained by the following method.

1 Cover film with a 1 per cent solution of Wollschwarz† for five minutes. This solution may be prepared by adding sulphuric acid to a stock 1 per cent solution of Wollschwarz as follows:

Wollschwarz 1 per cent (aqueous)	4.00 cc
Sulphuric acid 2 per cent	0.05 cc

2 Wash with water

3 Counterstain six to eight seconds with Loeffler's methylene blue which has been diluted with 15 parts of water. This acts as a mordant to the Wollschwarz, serving to fix it in the nucleus.

4 Wash with distilled water

5 Dry the film and examine under oil immersion lens

\*Received for publication November 10 1936

†Manufactured by G. Grubler & Co. Leipzig

automatically as the plunger is pulled, allowing fluid and air to enter the barrel of the syringe. The instant the outward movement of the syringe is stopped the check-valve becomes closed thus preventing the return of fluid or air to the pleural cavity. The moment this valve closes, another valve of similar design, located in the same housing, automatically opens. With the slightest forward movement of the plunger, air and fluid will be discharged through the valve, the connected nipple (*C*), and rubber tubing into any convenient receptacle. Two nipples connected with the housing of valve "*A*" are attachments for rubber tubing which lead to the manometer and thoracentesis needle, respectively.

The manometer (*F*), which is mounted on the same heavy metal base as the syringe, consists of nipple (*H*) for connecting the rubber tubing with valve

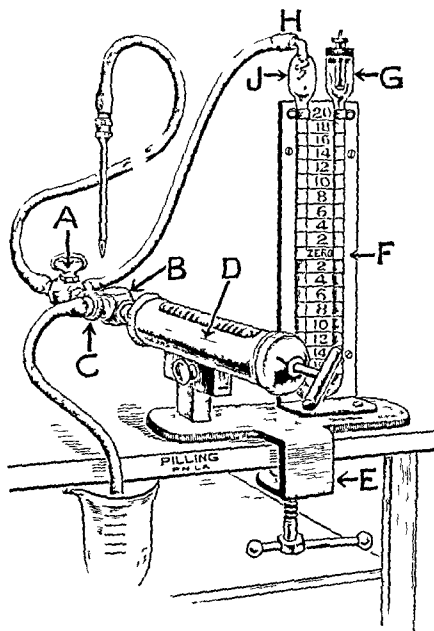


Fig. 1.—Aspirator showing syringe and manometer assembly.

"*A*," a spiral trap (*J*), and the plunger trap (*G*), the latter preventing the expulsion of fluid from the manometer. The manometer is of the standard type with unusual visibility of the numerals. The apparatus is fastened to a table by means of a swivel screw.

The apparatus is operated as follows: After the apparatus has been securely fastened to a table, the thoracentesis needle is introduced into the pleural cavity and valve "*A*" is turned to the right. In the presence of a pneumothorax the manometer will show oscillations and indicate the degree of intrapleural pressure; with fluid there will be no oscillations. Valve "*A*" is then turned to the left, and the syringe handle is placed perpendicularly and pulled slowly. With a pleural effusion the fluid will pass into the syringe chamber and the amount may be noted on the graduated scale. After filling,

after drying, this stain serves to readily distinguish spermatozoa by their distinctive morphologic characteristics and to clearly identify them from any foreign material that may be present.

If plenty of spermatozoa are present as they often are the examination need consume no more than five to ten minutes, and one obtains, as a result, a permanent preparation suitable for court evidence.

Another possible use to which such a test might be put is to identify the donor of a given semen sample. Because of the fact that the semen from a given individual presents characteristic spermatie anomalies occurring in constant ratios, it is possible to obtain sufficient information from a properly prepared semen smear to enable one to say positively whether another sample which is submitted for comparison was or was not derived from the same person.

#### SUMMARY

- 1 We have given a method for the positive identification of spermatozoa.
- 2 The microscopic examination not only differentiates spermatozoa of different species, but also may serve to identify different individuals of the same species.

455 BRIDGE STREET

## AN IMPROVED STAIN FOR USE IN RAPID BIOPSY DIAGNOSIS\*

A. L. BARBROW, M.D., PITTSBURGH, PA.

IN THE rapid diagnosis of biopsies by frozen section, anything which makes for speed and accuracy is worth while. For this purpose we have found the stain herein described eminently satisfactory. It is simple, rapid and sharply differentiating. It is a combination of Van Gieson's stain and Weigert's iron hematoxylin nuclear stain.

A	Hematoxylin	1 gm.
	95% alcohol	100 cc.
	This solution should be thoroughly ripened	
B	Liquor ferri sesquichlorati	4 cc.
	Hydrochloric acid	1 cc.
	Water	95 cc.
C	1% aqueous solution acid fuchsin	10 cc.
	Saturated solution picric acid	100 cc.

The stain is prepared by mixing these three solutions in the following proportions: 5 parts of A, 2 parts of B, and 10 parts of C. It is ready for immediate use. The frozen section is prepared in the usual manner; the slide is flooded with the stain for one minute and quickly rinsed in water. It may then be examined wet or after mounting in balsam. The mixture does not keep well and should be prepared fresh when needed.

2112 MURFAY AVENUE

\*From the Laboratories of Homestead Hospital, Homestead, Pa.  
Received for publication, October 13, 1936.

through "A" to within a few millimeters of the upper surface of the lower rubber stopper. The other end of stopcock "A" was connected by rubber tubing to a 100 c.c. syringe "C" which serves as the mercury reservoir. Mounted above the valve "A" was a three-way glass valve "D" of the type that permits any two outlets to be connected together or all three. The central arm is connected by short rubber tubing to a three-way glass connection "E." The side arm of this latter is connected to a manometer "F," and the lower by a rubber tubing to either "S," or "L," as will be explained subsequently. The other two outlets of the three-way valve "D" serve to interpose the whole apparatus between the source of sub- or supraatmospheric pressure and the apparatus, the pressure of which is to be controlled. The whole was mounted on a wooden panel with cut out circular areas so that the two lateral arms of the three-way valve "D" need not project beyond the board and to facilitate the slipping on of tubing connections. As an added convenience, a suction bottle was mounted behind the upright panel on the base board in a wooden vise (see Fig. 2).

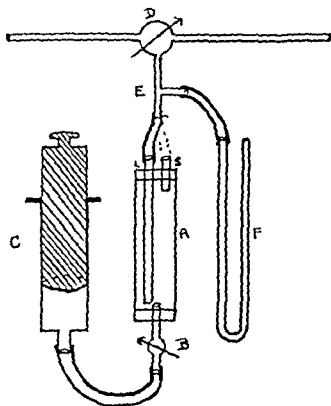


Fig. 1.

To place the apparatus in action, it is simply necessary to fill the reservoir "C" about two-thirds full of mercury. This is most conveniently done by removing the upper cork of the tube "A," opening valve "B" and exerting a slight pull on the syringe "C" while pouring mercury into "A." If the apparatus is to be used to control subatmospheric pressure, the rubber tubing leading from "E" is connected to the short tube "S" of the mercury valve "A." One of the side arms of "D" is connected to the pump with the suction flask interposed, if this be a water pump, and the other arm to the apparatus in which the subatmospheric pressure is to be maintained. The valve "D" is then turned so that the arm leading to the pump is connected to "A," while the arm connected with the apparatus is blocked off. The pump is then turned on. Mercury is allowed to flow slowly into the valve "A" by opening the valve "S" until the desired subatmospheric pressure is registered by the manometer "E." This will depend, as is apparent, on the height of the mercury column above the lower end of the tube "L." The stopcock "D" is then turned so that

They consist of a series of ten forms, one each for hematology, blood chemistry, urine, stomach contents, feces, body fluids, smears and cultures, serology, sputum, and basal metabolic rate. There is also a chart form on which the data from several days' laboratory work may be recorded. The

UNIVERSITY OF OREGON MEDICAL SCHOOL			
Room No. 206		OUTPATIENT CLINIC	
Case No. 14076		Body Fluids	
Name R., R.		Lab. No. 116556	
		Date 7/21/36	
Diagnosis <i>Meningitis</i>			
Source		✓ Cell count	151
Material	<i>Spinal fluid</i>	✓ P M N	28
Vol		✓ S L	72
✓ Appearance	<i>Clear</i>		
Sp. gravity			
✓ Globulin	<i>+</i>		
✓ Total Protein			
Spontaneous coag		<i>75 mg / 100 cc</i>	
Ordered by: <i>E. S. Roberts</i> Examined by: <i>Enter</i> Charted by: <i>R. D.</i>			

Fig 3—Color gray

UNIVERSITY OF OREGON MEDICAL SCHOOL			
Room No.		OUTPATIENT CLINIC	
Case No. 32905		Urine	
Name <i>F., J. L.</i>		Lab No. 116547	
		Date 7/1/36	
Diagnosis <i>Diabetes Mellitus</i>			
Routine	New pt.	Microscopic	Special
✓ Voided		Cas's Hyaline	✓ 24 hr vol <i>2020</i>
By catheter		Gran	✓ Sugar % <i>0.96</i>
Color	<i>Yellow</i>		✓ Gm 24 hr <i>17.37</i>
Appearance	<i>Sl. Cloudy</i>		Esbach (gm/L)
Reaction	<i>Acid</i>	Pus	<i>Occ.</i>
Sp gravity	<i>1.031</i>	Red cells	
Albumin	<i>-</i>	Crystals	
Reduction	<i>++</i>		Bile salts
Acetone	<i>+++</i>	Amorphous	<i>Urates +</i>
Diacetic	<i>+++</i>	Epithelium	<i>Occ.</i>
		Bacteria	
Ordered by: <i>J. C. E.</i> Examined by: <i>Joe</i> Charted by: <i>R. D.</i>			

Fig 4—Color, blue

forms are illustrated in Figs 1 to 12, Figs 11 and 12 representing the front and back of the chart form. Each of the forms represented in Figs. 1 to 10 is of a different color so that they can be easily picked out.

These forms (Figs 1 to 10) serve the purpose of an order form, a label for the specimen, a report form, and a record form. In addition, they have

The length of the chamber "A" should be roughly twice that of the greatest sub- or supraatmospheric pressures expressed in height of mercury column for which the apparatus is to be used. The glass tubes "S" and "L" should not differ appreciably in bore from the *narrowest* bore interposed between the apparatus and the source of sub- or supraatmospheric pressure.

The above apparatus may be put to many uses. A few may be mentioned: filtration, boiling, or evaporation under controlled, moderately reduced pressure; regulation of aeration in urea determinations; the regulation of gas pressures in tonometers; the continuous evacuation of air or fluids from body cavi-

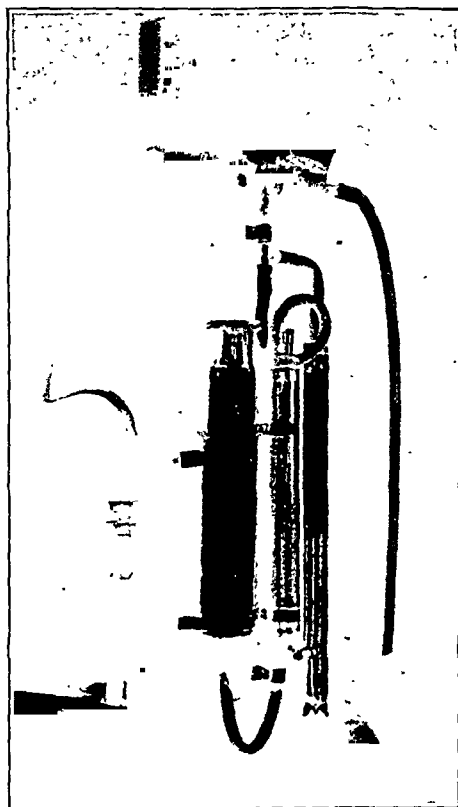


Fig. 3.

ties (e.g., ball valve or leaking spontaneous pneumothoraces, etc.) or from hollow visci (dilatation of the stomach, inflated bowels) under conditions such that the patient is protected against sudden fluctuations of the pump, or its accidental manipulation; the withdrawal of large quantities of blood from a vein under slight subatmospheric pressures (as in indirect transfusion), et cetera. A similar apparatus has been used in this laboratory for artificial respiration in open chest experiments in dogs. In this instance, a three-way valve was inserted between the trachea of the animal and the apparatus, the degree of pulmonary inflation being easily controlled by variation in the pressure head, and the rate of respiration being controlled by turning the valve by hand, the dog

the slip is received at the laboratory the name and case number are entered in a book together with a laboratory number, which is stamped on the slip and in the book, and check marks are made in this book under the name of the material and the tests wanted. The statistics of the laboratory are compiled by adding these checks.

## UNIVERSITY OF OREGON MEDICAL SCHOOL

## OUTPATIENT CLINIC

Room No

Case No 2104

Hematology

Name H. R.

26563

Lab No

Date 7/20/36

Diagnosis *Perniciou anemia*

Source

✓ Routine	Special	Special
Hb % 37.6	✓ Cell Vol 14.12	Platelets
Gm 5.19	✓ Sed Rate 15 min 7	Bleed Time
R B C 1.14	45 min 26	Cot Retr Time
W B C 5,150	✓ Reticulo 6.0%	Coag Time
No Counted 100	✓ Col I 1.52	Malaria
P M N 68	✓ Vol I 1.48	Aniso +
St Cells 1	✓ Sat I 1.03	Poikilo
P M E 1	✓ Ict I 7	Polychrom +
P M B	Van den Bergh	Macro. +
S L 24	Fragility, begin	
Mon 6	Fragility, complete	
Disin cells		

Ordered by H. R. Lee

Examined by See

Charted by R'D

Fig 7—Color pink

## UNIVERSITY OF OREGON MEDICAL SCHOOL

## OUTPATIENT CLINIC

Room No

Case No 26301

Feces

Name J. H. P

26566

Lab No

Date 7/20/36

Diagnosis *Amoebic Dysentery*

Routine	Occult blood only
✓ Color Brown blood streaked	Element
Odor	✓ Ameba <i>Endameba histolytica</i> +
Consistency Liquid	✓ Flagellate None found
Occult blood +	✓ Worms None found
Mucus +	✓ Ova None found
Pus —	

Ordered by Dr Shaw

Examined by C. Rice

Charted by R. D.

Fig 8—Color dark yellow

When the tests are performed in the laboratory, all calculations are made on the back of the slip and the results are recorded in the space after the test, as illustrated. The technician doing the work initials the slip and the results are then recorded on the patient's chart (Figs 11 and 12) and the slip is initialed by the person doing the charting, then returned to the laboratory.

This stain imparts to essentially everything aside from the heads of the spermatozoa a dull grayish color, and the heads, standing out in this drab background, will be seen as bright golden or yellowish spots as plain as a beacon

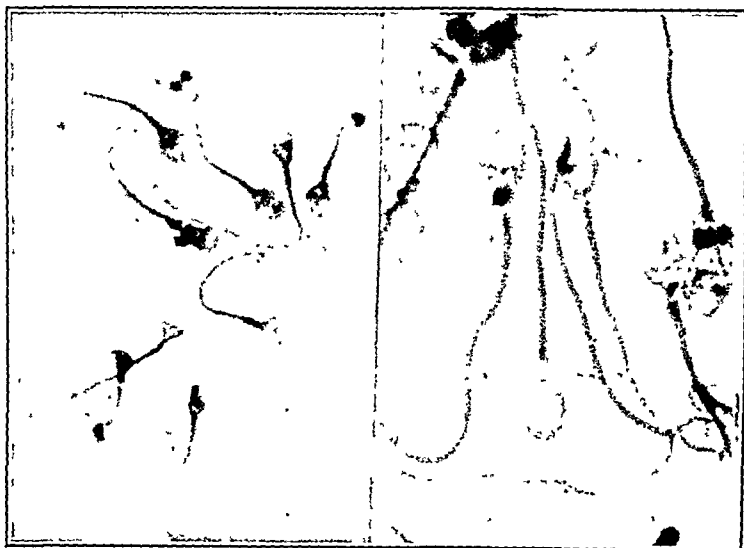


Fig. 1.—Photomicrographs of spermatozoa from normal population. Note the uniformity of size and contour. (Wollschwarz stain.)

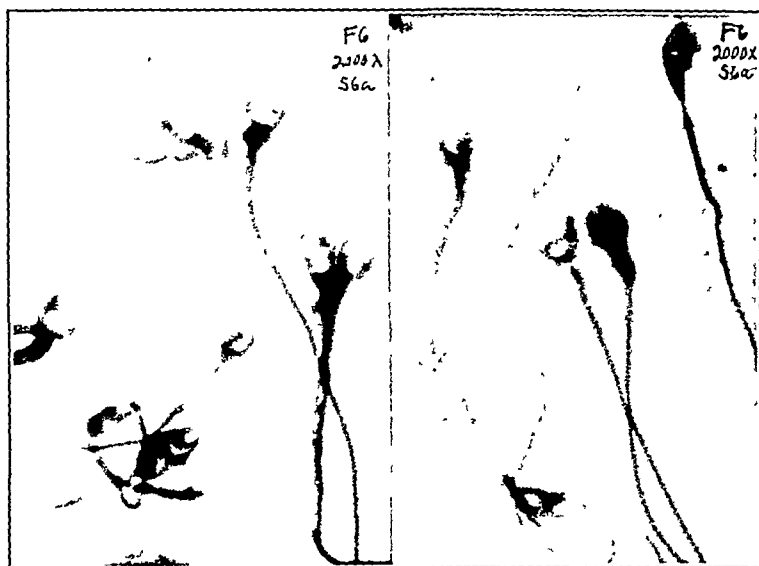


Fig. 2.—Photomicrographs of spermatozoa from pathologic population. Microspermia and various odd-shaped cells abound. (Wollschwarz stain.)

in the night. This stain is exceptionally selective for spermatozoa. With it the nuclei of tissue cells may also be stained, but here the morphology is so different that there needs to be no confusion. Because of the fact that the spermatozoon head maintains its morphologic characteristics rather indefinitely



# A SIMPLE SET OF LABORATORY FORMS\*

EDWIN E. OSGOOD, M.D., PORTLAND, ORE.

FOR the benefit of other laboratories, it seems worth while to describe a set of forms which have been in use in the University of Oregon Medical School since 1931. They have been copied by many other institutions and have proved to be labor saving to both the physician and laboratory worker.

UNIVERSITY OF OREGON MEDICAL SCHOOL 26559  
 Room No. OUTPATIENT CLINIC  
 Case No. 621  
 Name N., R. H. Sputum Lab. No.  
 Date 7/20/36

Diagnosis <i>Bronchial Asthma</i>	
Routine	✓ Curschman's spirals +
Hrs.	
Vol.	Vincent's Organisms
Color	Pneumococci
Odor	Streptococci
Pus	T. B. Concentration
Elastic fibres	
Layers	✓ Eosinophils #
B. tuberculosis	Charcot-Leyden cryst.

Ordered by *C. Vetch* Examined by: *E. Rice* Charted by: *R. D.*

Fig. 1.—Color, cream.

UNIVERSITY OF OREGON MEDICAL SCHOOL 116555  
 Room No. OUTPATIENT CLINIC  
 Case No. 10776  
 Name T., J. Stomach Contents Lab. No.  
 Date 7/21/36

Diagnosis <i>Ca of stomach</i>	
Meal <i>Ewald</i>	Total acid 2
Time <i>1 hr.</i>	Lactic acid +
Vol. <i>15 cc.</i>	Occult Blood +
Appearance <i>yellow</i>	
Free HCl <i>None</i>	Boas-Oppler bacilli +
Boas HCl <i>Neg.</i>	Sarcinae —

Ordered by: *H. A. Brown* Examined by: *E. Rice* Charted by: *R. D.*

Fig. 2.—Color, white.

\*From the Department of Medicine, University of Oregon Medical School.  
 Received for publication, July 28, 1936.

the slip has been filed. Since the slips are of paper and small in size compared to most record forms, they take up very little room yet in an experience with over 300,000 tests, they have proved adequate.

The chart form has the great advantage that the physician can tell at a glance whether a laboratory finding is changing by merely glancing across the form instead of thumbing through many pages as is necessary with many types of laboratory records. The laboratory sheet is of a different color from the other pages of the patient's chart so that one can find it easily.

#### SUMMARY

A simple set of laboratory forms is described, which have the following advantages: (1) They save writing for the physician, the technician, and the laboratory clerk. (2) One form serves the purpose of four forms: an order form, a label for the specimen, a report form, and a record form. (3) They prevent arguments. They have demonstrated these advantages in actual use in a laboratory where more than 300,000 determinations have been done since their use was inaugurated.

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## LIPOLYTIC ACTIVITY OF THE LACTOBACILLUS ACIDOPHILUS\*

DAVID B. SABINE, B.S., YONKERS, N. Y.

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A SIMPLE method for the detection of microbial lipase, described by Berry,<sup>1</sup> can be utilized for the differentiation of strains of the *Lactobacillus acidophilus*. The test consists of streaking the culture on agar to which butter fat has been added. The lipolytic activity is shown by the formation of bluish green insoluble copper soap which appears when the plate following an appropriate incubation period, is flooded with a saturated solution of copper sulphate. In the following experiments, Torrey's liver glucose agar, as recommended by the author,<sup>2</sup> was used.

Of a collection of 19 strains of *L. acidophilus* isolated from many different sources, 7 failed to show any lipolytic activity. The data are shown in Table I.

With the exception of Strains 3,266 and 3,269, which failed to ferment sucrose and maltose, all the strains fermented dextrose, maltose, sucrose, levulose, lactose, and galactose. The only method of differentiation lay in morphology and colonial form. In several cases these were similar, making classification difficult. Since this situation often arises, it was hoped that the lipolytic reaction might prove of assistance.

A striking example of the value of the test is illustrated by Strains 180 L and 180 S. They were isolated from the same child and their cultural and morphologic characteristics were nearly identical. However, a separation based

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\*From the Laboratories of the Arlington Chemical Company.  
Received for publication November 19, 1936.

the advantage that the final record is all in the original writing of the person ordering or doing the work or charting, so that there can be no argument as to who was at fault if any error occurs.

The manner of using these forms is very simple. The physician ordering a test fills in the room number, case number, name of patient, the date, the

UNIVERSITY OF OREGON MEDICAL SCHOOL		116545
Room No.	OUTPATIENT CLINIC	Lab. No.
Case No. 7431	Basal Metabolic Rate	Date 7/1/36
Name F., R.		
Diagnosis		
Age 36	Bar. Pres 748.5	PS24-13187-1-33-2M
Height 64 3/4 inches	Temp 21.0	
Weight 208 pounds	Cc per min. 248	
Pulse 56	Rate -12.2	
Temp. 98.6		
Cooperation Good		
Ordered by: H. Round		Examined by: S.
		Charted by: R.D.

Fig. 5 —Color, light yellow.

UNIVERSITY OF OREGON MEDICAL SCHOOL		
Room No.	OUTPATIENT CLINIC	26562
Case No. 19041	Serology	Lab. No.
Name R., J. W.		Date 7/20/36
Diagnosis Paresis		
Material Spinal fluid	Widal	
✓ Kolmer +++		
✓ Kahn +++	Blood Group.	
✓ Lange 5554320000	Matching	
Ordered by: G. R. Olsen	Examined by: Somes	Charted by: R. D.

Fig 6.—Color. green

tentative diagnosis, and checks such tests as he wishes to order, checking merely routine, to include all the routine procedures on that type of specimen. He then initials the slip and if he takes the specimen himself, gums the slip to the specimen as a label; otherwise, he sends the slip to the laboratory as an order, and as the specimen is taken the slip is attached as a label. When

PC27-15269-1 36-12M

described by Spray<sup>3</sup> in 1930 was advocated for use in culturing anaerobes by the alkaline pyrogall method, and we have had excellent results with the technique described by him. The rapidity and ease with which individual cultures could be grown prompted us to use this type of plate to grow cultures under increased carbon dioxide tension.

#### METHOD

As described by Thompson.<sup>2</sup> The two solutions to produce carbon dioxide are (1) a molar solution of sodium bicarbonate (84 gm to one liter of distilled water), (2) a solution of sulphuric acid (1 cc of concentrated acid added to 29 cc of distilled water). The sulphuric acid does not need sterilization but the sodium bicarbonate should be boiled or autoclaved before use. These solutions when mixed in equal parts liberate 22.4 cc of carbon dioxide for each cubic centimeter of bicarbonate solution. Since the Spray anaerobic culture dish has a capacity of something over 200 cc 1 cc of the bicarbonate solution is, therefore, sufficient to create a carbon dioxide concentration of approximately 10 per cent. Solutions are introduced into each side of the partition of the base of the dish with a 1 cc pipette. The Petri dish containing the desired medium is placed over the base and sealed with melted paraffin. The assembled dish is then tilted slightly and solutions are well mixed. Care should be taken to allow the paraffin to harden completely before the two solutions are mixed.

#### DISCUSSION

This combination allows a quick, easy method of preparing individual cultures. The dish is easily inspected without breaking the seal. In our hands enough growth of *Neisseria intracellularis* has been obtained within sixteen hours from spinal fluid in sufficient quantities to obtain test agglutination with specific therapeutic serum. *Neisseria gonorrhoeae* can be isolated in twenty-four hours from vaginal swabs. Specific colonies are picked by means of the McLeod<sup>1</sup> oxydase method.

In one instance cultures of *Neisseria intracellularis* were readily obtained from the blood of a patient showing the clinical symptoms of meningococcus septicemia. Blood cultures by this method should be of particular value when an atmosphere of 10 per cent carbon dioxide is desired.

The extraordinary variety of organisms as well as profusion of growth from cultures of vaginal swabs suggest that this should be a most valuable method of studying this particular group of vaginal flora.

#### REFERENCES

- 1 McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B. Cultivation of the Gonococcus as a Method in the Diagnosis of Gonorrhoea With Special Reference to the Oxydase Reaction and to the Value of Air Reinforced in Its Carbon Dioxide Content, *J. Path. & Bact.* 39: 221, 1934.
- 2 Thompson, Luther. A Simple Method of Supplying Carbon Dioxide in Jars for Bacteriologic Cultures, *Am. J. Clin. Path.* 5: 313, 1933.
- 3 Spray, Robert Spalding. An Improved Anaerobic Culture Dish, *J. Lab. & Clin. Med.* 16: 203, 1930.

where these slips are filed in a file according to the institution, a drawer according to the material, alphabetically under the patient's name by current month and current year.

Should a physician state that he ordered a test that is not on the chart, a glance at the slip will show his initials and if there is no check mark by the

UNIVERSITY OF OREGON MEDICAL SCHOOL		
OUTPATIENT CLINIC		
Blood Chemistry		
Room No.		26556
Case No.	60/45	Lab. No.
Name	J., R.	Date 7/20/36
Diagnosis <i>Glomerular Nephritis</i>		
<input checked="" type="checkbox"/> Urea N.	75 mg./100 cc.	Cholesterol
<input checked="" type="checkbox"/> Creatinine	4.5 mg./100 cc.	Phosphate
<input checked="" type="checkbox"/> Alkali Reserve	29.1	Serum Proteins
Calcium		
Dextrose		
		Chlorides
		Uric Acid
		N. P. N.
Ordered by: <i>J. Roberts</i> Examined by: <i>Jee</i> Charted by: <i>R.D.</i>		

Fig. 9.—Color, rose.

UNIVERSITY OF OREGON MEDICAL SCHOOL		
OUTPATIENT CLINIC		
Smears and Cultures		
Room No.		26555
Case No.	26/10	Lab No.
Name	H., S.	Date 7/20/36
Diagnosis <i>Tonsillitis</i>		
Source	<i>Throat</i>	G. P. Inoculation
Material	<i>Pus on swab</i>	
Examine For		
<input type="checkbox"/> B. Tuberculosis		Pneumococcus
<input type="checkbox"/> T. B. Concentration		Typing
<input type="checkbox"/> Vincent's Organisms		Gram Neg. Intrac. Dip.
<input checked="" type="checkbox"/> Diphtheria	<i>None found</i>	
Smear		
<input checked="" type="checkbox"/> Culture	<i>Pure culture - Strept. hemolyticus</i>	
Ordered by: <i>M. Carleton</i> Examined by: <i>D. Roe</i> Charted by: <i>R.D.</i>		

Fig. 10.—Color, buff.

test, he has nothing further to say. If there is a check mark and a test is not on the slip, the initials of the technician indicate who is responsible. If the result is on the slip but not on the chart, the initials of the person charting indicate responsibility here. A check mark is made in the laboratory book when the slip is returned to the laboratory so that one knows at once whether

proportion of 1 gram of powder to 5 c.c. of alcohol. This suspension is shaken by hand or in a mechanical shaking apparatus for one and one half hours and then filtered immediately through a dense filter paper. The alcoholic extract is measured, and 0.6 per cent cholesterol is added. This is dissolved by shaking in a glass stoppered bottle or by rotating the bottle in a water bath at about 50° C. for ten minutes. The cholesterolized extract is filtered. The antigen is then ready for use. It is stored in a brown glass stoppered bottle at room temperature and protected against light. This antigen will remain stable for many months.

#### EMULSIFICATION AND DILUTION OF THE ANTIGEN

Into a large test tube is measured 0.6 c.c. of 0.45 per cent sodium chloride solution. To that is added slowly 0.7 c.c. of 1 per cent cholesterol in absolute alcohol. This is rotated lightly for a few seconds. Then 0.6 c.c. of antigen is added slowly and the mixture is shaken vigorously for about one minute. The emulsion is diluted with 2.5 c.c. of 0.9 per cent saline, and the tube is again shaken vigorously for one minute. The antigen emulsion is then ready for use. For this method special titration is not required and the emulsion will remain stable for about forty eight hours at a temperature of 20° C.

#### PERFORMANCE OF THE TEST

The size of the tube should be approximately 9 mm (inside diameter) by 75 mm. The unit of serum is 0.15 c.c. If citrated whole blood is used, the unit is 0.2 c.c.

1 The unit of unheated serum or freshly citrated whole blood is measured into the respective tubes.

2 To each tube is added 0.05 c.c. of half saturated ammonium sulphate. This is mixed by shaking the rack for a few seconds.

3 Into each tube is then measured 0.05 c.c. of antigen emulsion, and the rack is again shaken to mix the contents.

4 The tubes are centrifuged at the rate of about 2,000 revolutions per minute for three minutes. If that velocity cannot be obtained, the time is extended accordingly.

5 The tubes are removed from the centrifuge and to each is added 3 c.c. of distilled water. By placing the index finger over the mouth of the tube, it is inverted twice without shaking the contents. This is important. If whole blood is used for the test, the fluids are, without inverting the tubes, poured into other tubes with identical numbers, leaving the blood cells at the bottom of the first tube, which can then be discarded.

6 All tubes with the flocculate or the emulsion suspended in 3 c.c. of distilled water, are again centrifuged at the rate of about 2,000 revolutions per minute for two to three minutes, thus collecting the sediment at the bottom of the tubes.

7 After the tubes have been removed from the centrifuge, the fluid content is decanted by completely inverting a handful of tubes, allowing damage to the last drop. The inverted tubes are shaken once to insure complete damage.

Fig. 12.—Color, orange.

tive tests consist of unflocculated emulsion. Even weak positive reactions are important as false positive tests are very rare. A special lamp for reading the results may be of value to the inexperienced operator.

#### RESULTS OBTAINED

By this method 5 000 blood tests and over 500 spinal fluid reactions have been compared with several other methods of known relative value. Among the blood samples were over 500 from patients in various stages of syphilitic infection. In cases of treated syphilis this method proved of equal value to the test previously reported,<sup>1</sup> which is one of the most sensitive reactions in treated cases of syphilis. In congenital and primary syphilis this centrifugation method proved to be more sensitive.

On spinal fluid this method proved to be more sensitive and more reliable than the Wassermann reaction as performed in our laboratory.

#### SUMMARY

1. A simple and reliable centrifugation method for the diagnosis of syphilis has been described.

2. This method appears to be as sensitive and as specific as any syphilis test used in this country.

3. For this centrifugation method unheated serum and citrated whole blood are satisfactory.

4. This test can be performed with very little equipment as the water bath, shaking apparatus, special lamps and hand lenses are not required.

5. The antigen is simple to prepare and will remain stable for many months.

6. The test requires but a few minutes for its performance.

#### REFERENCES

1. Rytz, F. A Rapid Flocculation Method for the Diagnosis of Syphilis. Technique for Spinal Fluid, J LAB & CLIN MED 22: 82, 1936.
2. Cumming, H. S., et al. The Evaluation of Serodagnostic Tests for Syphilis in the United States, J A M A 104: 2083, 1935.
3. Rytz, F. A Rapid Flocculation Method for the Diagnosis of Syphilis, J LAB & CLIN MED 21: 934, 1936.

on a slight difference in colony size and a difference in the time of the fermentation reactions was effected. Later, when the lipase test was applied, a clear-cut differentiation was obtained.

TABLE I

NO.	SOURCE	COLONY TYPE	LIPOLYTIC ACT
3264	Commercial concentrate	Y	Positive
3262	Adult's feces	Y	Positive
5	Adult's feces	Y	Positive
B-330	Adult's feces	Y	Positive
3268	Commercial culture	Y	Positive
180 S	Child's feces	Y	Positive
180 L	Child's feces	Y	Negative
A	Commercial concentrate	Y	Positive
M	Child's feces	Y	Positive
3010	Child's feces	Y	Positive
3272 B	Commercial culture	Y	Positive
3266*	Commercial culture	X	Positive
3017 B	Commercial acidophilus milk	X	Negative
3017 A	Commercial acidophilus milk	X	Negative
3270	Commercial acidophilus milk	X	Negative
3269†	Commercial culture	X	Negative
3272 A	Commercial culture	X	Negative
3267	Commercial acidophilus milk	X	Positive
MF	Infant's feces	X	Negative

\*Commercial culture of *L. bulgaricus*.

†Labelled acidophilus but its cultural characteristics were more closely allied to *bulgaricus*.

In clinical work, this test has already proved of value in demonstrating that a strain administered was identical with the strain recovered.

## REFERENCES

1. Berry, J. A.: Detection of Microbial Lipase by Copper Soap Formation, *J. Bact.* 25: 433, 1933.
2. Sabine, D. B.: A Comparison of Media for Plating *L. Acidophilus*, *J. LAB. & CLIN. MED.* 21: 843, 1936.

## INDIVIDUAL CULTURE DISH WITH INCREASED CARBON DIOXIDE TENSION\*

AUSTIN L. JOYNER, AND CLAUDIUS P. JONES, DURHAM, N. C

THE use of carbon dioxide to stimulate the growth of certain bacteria has been given particular attention in recent years. McLeod<sup>1</sup> emphasized the importance of carbon dioxide in his description of methods for isolating *Neisseria gonorrhoeae*, and Luther Thompson<sup>2</sup> described a simple method of introducing this gas in measured quantities into culture jars. These methods, in our experience, not only increase the accuracy of diagnosis but very appreciably shorten the time required to demonstrate the organisms.

The purpose of this paper is to call attention to the advantages of the above methods when applied to the Spray anaerobic culture dish. This dish,

\*From the Department of Bacteriology and the Department of Obstetrics and Gynecology of Duke University School of Medicine.

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In order to obviate the possibility of an contamination while dispensing sterile filtrates, a flask has been designed which combines the essential features of an aspirator bottle and an ordinary filter flask. Fig. 1 indicates how the flask is employed with a Berkefeld candle and a glass bell filling device for filtering and dispensing culture media, sugar solutions, etc.\* This apparatus practically precludes any possibility of an contamination in dispensing sterile filtrates.

To prepare the flask for use either a Berkefeld or Seitz filter is fastened in the top of the flask with a single hole rubber stopper. A strip of cotton is fastened around the joint between the rubber stopper and the lip of the flask to prevent contamination should the stopper become loose in handling. The side tubulation may be plugged with cotton or connected to a glass bulb stuffed with cotton as shown in the figure. A glass bell filling device, completely covered with tin foil or heavy paper is connected to the lower tubulation with a short piece of thin walled rubber tubing. The entire apparatus is then placed in the autoclave for sterilization.

After the apparatus has cooled, it is placed on a ring stand as shown in the figure. The side tubulation is connected with the source of vacuum and a pinch clamp is fastened on the rubber tubing just below the lower tubulation. The material to be filtered is poured into the filter and the vacuum turned on. When a sufficient amount has been filtered, the vacuum is turned off, the covering removed from the bell filling device and test tubes, flasks, etc., are filled by inserting them inside of the filling device and releasing the pinch clamp. The usual precautions of flaming the tubes before and after filling should be observed with an occasional flaming of the mouth of the filling device.

This apparatus finds many uses in the routine and research bacteriologic laboratory where culture media, toxin broths, serum, sugar solutions, etc., must be sterilized by filtration and aseptically dispensed. In our laboratory this apparatus is chiefly used for filtering large quantities of serum used in the preparation of various types of media.

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\*The flask and filling device may be obtained in various sizes from the Corning Glass Works, Corning, N. Y.

# A SIMPLE CENTRIFUGATION METHOD FOR THE DIAGNOSIS OF SYPHILIS\*

F. RYTZ, MINNEAPOLIS, MINN.

IN THE present centrifugation method for the diagnosis of syphilis are embodied the same principles for blood as earlier described for spinal fluid.<sup>1</sup> This method requires very little equipment, a minimum of technical skill, and only a few brief moments for its performance. It is based on the importance of the specific gravity in relation to the relative surface tension of the serum-antigen mixture. The depth of the serum-ammonium sulphate-antigen mixture in the test tube must approximate the inside diameter of the tube. If the depth of the serum-sulphate-antigen mixture in the test tube used is greatly out of proportion to the diameter of the surface, the latter being relatively decreased, the combined antigen-antibody will not, during the process of centrifugation, be forced to the surface as desired, and the combination is then too infirm for further manipulation of the flocculate. The test would at the same time be less sensitive. For that reason the diameter of the test tube must be in proportion to the amount of serum used, and that amount in turn dictates the quantity of antigen emulsion, which is equal to the amount of half saturated ammonium sulphate: 1 part of the emulsion to 3 parts of the serum. By that combination the specific gravity is at the same time brought to a desirable point, and this technique eliminates entirely the heating of the serum or the blood.

Both citrated whole blood and unheated serum are satisfactory for the test. In cases of emergency, it is most convenient to use freshly citrated, unheated whole blood, but the test must then be performed within two hours after collecting the sample. If the test cannot be performed within that time, unheated serum is preferable. For this method, if serum is used, it is important to avoid hemolysis.

## PREPARATION OF THE ANTIGEN

Fifty grams of Difco beef-heart powder are placed in 300 c.c. of anesthesia ether, contained in a 1 liter Erlenmeyer flask, and shaken for five seconds every ten minutes for one hour. The suspension is then filtered through a dense filter paper and the powder allowed to dry completely by spreading it out on the filter paper. The dry powder is again placed in 1 liter flask to which is added 300 c.c. of pure acetone. This is shaken for five minutes and then filtered as before. To free the powder from the last drops of acetone, it is pressed lightly with a tongue blade covered with tin foil. In order to dry completely, the powder is placed in the incubator for three to six hours. The dried powder is then weighed and suspended in absolute ethyl alcohol in the

\*From the Clinical Laboratories of the Minneapolis General Hospital.  
Received for publication, December 7, 1936.

## METHOD

Freshly drawn clotted blood is centrifuged and 1 cc of serum is transferred to a graduated centrifuge tube. An equal quantity of colorless redistilled acetone is added. In the case of deeply jaundiced serums three parts of acetone are added. The solution is mixed, allowed to stand for five minutes, and centrifuged. The supernatant fluid is pipetted off into a standard sized test tube and compared with the standards in a colorimeter box. For further dilution, if necessary, acetone is used. The same light source preferably a strong daylight lamp should be used for all determinations.

Standards are made up every few months and frequently checked against freshly prepared solutions. Ten standards of from one part to ten parts in 10,000 of potassium dichromate in distilled water are required. These are placed in the standard sized test tubes, a drop of 0.1 N hydrochloric acid is added to each, and the tubes are then sealed.

Calculation: Icteric index = number of the standard used  $\times$  dilution of original serum. For example, if a serum to which 3 parts of acetone were added matches standard No. 8 (eight parts of dichromate in 10,000) the icteric index is  $4 \times 8 = 32$ .

TABLE I

NUMBER OF OBSERVATIONS	MG BILIRUBIN BY QUANTITATIVE VAN DEN BERGH (MG PER CENT)	ICTERIC INDEX BY ACETONE METHOD			ICTERIC INDEX BY WATER METHOD		
		AVERAGE	LOWEST AND HIGHEST VALUES	MEAN VARIATION FROM AVERAGE	AVERAGE	LOWEST AND HIGHEST VALUES	MEAN VARIATION FROM AVERAGE
114	0.1-0.5*	3.7	1.10	1.6	10.4	4.35*	4.0
34	0.6-1.0	9.1	4.15	2.1	24.7	8.65	11.3
13	1.1-1.5	13.8	10.20	1.9	39.1	23.75	9.7
16	1.6-2.5	17.6	12.40	3.8	34.5	22.65	9.9
15	2.6-5.0	24.7	15.36	5.6	66.7	28.10	19.1
30	5.1-16.0	39.6	19.70	10.0	120.6	40.18	30.4

\*NOTE: Within the normal range (0.1-0.5 mg per cent) of the quantitative van den Bergh reaction the icteric index by the acetone method was greater than five in 20 cases and greater than six in 12 cases; there were 38 values above ten (upper limit of normal) by the water method in this same range.

## RESULTS

The table shows the variations and average values of the icteric index for various ranges of bilirubin concentration by the van den Bergh method. In each of the 222 analyses the quantitative van den Bergh reaction and the icteric index by both the saline\* and the acetone methods were done. The table indicates that the acetone method gives values varying within a much narrower range than does the saline method for the same quantity of bilirubin by the van den Bergh test. The acetone method yields results averaging about one third to one half those obtained by the saline method. However, when serums contain over 5 mg per cent of bilirubin, the icteric indices do not bear a constant relationship to the van den Bergh. Although by the water

\*The saline method is similar to the acetone method except that precipitation of proteins is omitted and serums are compared directly with the standards. Dilution when necessary is carried out with normal saline (0.9 per cent) or distilled water.

8. To the sediment of each tube is added 1 c.c. of 0.9 per cent saline. Before reading the results, each tube is inverted once by placing the index finger over the mouth of the tube.

9. The results are easily estimated against a favorable light without the aid of special facilities. In negative results, the emulsion scatters readily by inverting the tube. A positive test has large floccules in a crystal clear fluid. A weakly positive reaction consists of smaller floccules in a slightly hazy fluid. Weak reactions should be repeated on the same sample, and, as with any test, checked by one or two other methods of known relative value.<sup>2</sup> The test previously described by the authors<sup>3</sup> may conveniently be used for such checking, as highly decomposed blood is satisfactory for that method, and the antigen for the present test is satisfactory for both methods.

#### SPINAL FLUID

Except for the difference in antigen, which permits omission of the three-minute shaking, the technique for spinal fluid is identical with the test previously reported.<sup>1</sup> The fluid should not be over three days old. Traces of blood render the spinal fluid unsatisfactory for the test. A cloudy fluid should be centrifuged before performance of the test. For the spinal fluid test, the emulsion should be prepared not more than one hour before use.

1. Of unheated spinal fluid 0.2 c.c. is measured into the respective tubes of the same size as described for the blood test.

2. To each tube is added 0.1 c.c. of half saturated ammonium sulphate. This is mixed by shaking the rack vigorously for a few seconds.

3. Then 0.1 c.c. of half saturated sodium chloride is added, and again the rack is shaken to mix the contents.

4. To each tube is measured 0.05 c.c. of antigen emulsion. This is mixed by shaking the rack.

5. The tubes are then centrifuged at the rate of about 1,500 revolutions per minute for fifteen minutes. If that speed cannot be obtained, the time is extended accordingly.

6. After the tubes have been removed from the centrifuge, 3 c.c. of 0.45 per cent saline are added to each tube, and all tubes, one by one, are inverted 10 times by placing the index finger over the mouth of the tube.

7. The tubes are again centrifuged, at the rate of about 2,000 revolutions per minute, for three minutes, collecting the floccules or the emulsion at the bottom of the tubes.

8. The fluid content is decanted in the manner described for the blood test, allowing complete drainage of the tubes by careful manipulation to retain the sediment at the bottom of each tube.

9. To each tube is added 1 c.c. of 0.9 per cent saline. Before reading, each tube is inverted slowly once or twice without shaking the contents.

10. The results are estimated against a favorable light. The floccules from spinal fluid are somewhat finer than those resulting from the blood test. A strongly positive reaction shows fairly large floccules in a clear fluid. A weakly positive reaction has smaller floccules in a slightly hazy fluid. Nega-

My thanks are due to Dr Louis J Soffer and Dr Harry Sobotka for suggesting this investigation

## REFERENCES

- 1 Ernst, Z, and Forster, J Über die Bestimmung des Blutbilirubins Klin Wchnschr 3 2386, 1924
- 2 Van den Bergh, A A H Die Gallenfarbstoffe im Blute Leipzig 1918  
Van den Bergh, A A H, and Grotepas, W An Improved Method for Determining Bilirubin in Blood, Brit M J 1 1157 1934
- 3 Meulengracht, E Die klinische Bedeutung der Untersuchung auf Gallenfarbstoff im Blutserum, Deutsches Arch f Klin Med 132 255 1920
- 4 Nation, E, and Myers, V Carotinemia Proc Soc Exper Biol & Med 31 620, 1934
- 5 Soffer, L J Present Day Status of Liver Function Tests, Medicine 14 185, 1935

## A CRITICISM ON THE SUMNER METHOD FOR URINE SUGAR\*

SAUL MALKIEL, M A, BOSTON, MASS

USE of the Sumner method for urine sugar indicated that normal urine constituents might interfere with the sugar analysis

Solutions were prepared containing certain constituents of normal urine, glucose to known concentration added, and recovery attempted by the dinitrosalicylic acid reagent method of Sumner<sup>1</sup> The reagent and method utilized were such as described<sup>2</sup> and later modified<sup>1, 3</sup> by Sumner

Typical results as to the percentage of glucose recovered may be tabulated as shown in Table I

TABLE I

SOLUTION INGREDIENTS	MG ADDED	GLUCOSE RECOVERED	PER CENT RECOVERY
Urea	2.00	1.46	73.0
Urea	2.00	1.79	89.5
Urea	2.00	1.72	86.0
Uric acid	2.00	1.70	85.0
Uric acid	2.00	1.66	83.0
Uric acid	2.00	1.72	86.0
Creatinine	2.00	1.40	70.0
Creatinine	2.00	1.62	81.0
Creatinine	2.00	1.60	80.0
Urea, uric acid, creatinine	2.00	0.91	45.5
Urea, uric acid, creatinine	2.00	0.89	44.5
Urea, uric acid, creatinine	2.00	0.91	45.5
Urea, uric acid, creatinine, NaCl	2.00	0.89	44.5
Urea, uric acid, creatinine, NaCl, CaCl <sub>2</sub>	2.00	0.83	41.5

To simulate normal urine the above constituents were added in these physiologic amounts: urea, 20 mg/ml, uric acid, 0.5 mg/ml, creatinine, 1 mg/ml, sodium chloride, 10 mg/ml, and calcium chloride, 0.5 mg/ml

The above analyses without the added glucose were also simultaneously carried out as controls and compared to a standard of blank reagent. Reduction to some degree was observed in these cases

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## A FILTER FLASK FOR DISPENSING FILTRATES ASEPTICALLY\*

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THE usual method of dispensing filtrates after they have passed through a Seitz or Berkefeld filter into the filter flask is by pipetting or pouring the filtrate out of the flask. It is obvious why filtrates dispensed in this manner frequently become contaminated with air-borne microorganisms during the

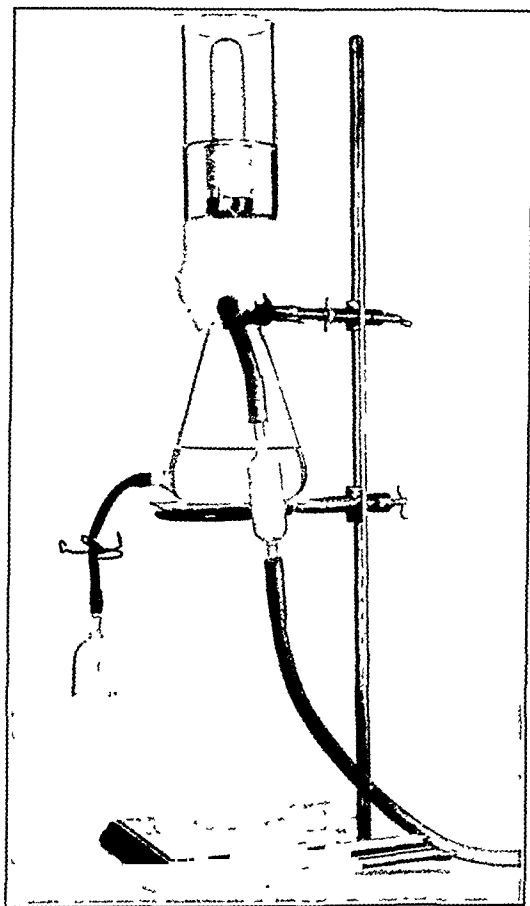


Fig. 1.

process. Those who have worked with or who are familiar with the literature on filtrable forms of bacteria are too well acquainted with air contaminants as a possible source of growth in filtrates and the consequent questionable nature of any "filtrable forms" obtained.

\*From the Division of Laboratories, Illinois State Department of Public Health  
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## CHEMICAL TESTS FOR THE DIAGNOSIS OF PREGNANCY\*

ISADORE GERSH, M D AND JULIAN LEWIN B A DENVER COLO

THE possibility of diagnosing pregnancy by chemical tests has interested the medical profession during the past few years. In an editorial of a recent issue of the *Journal of the American Medical Association*<sup>1</sup> the diagnosis of pregnancy by means of the bromine test for urinary histidine was discussed. It was pointed out that the usefulness of the test is not definitely known and further investigation was recommended to determine the value and significance of it. Another chemical test which also has aroused much interest was suggested by Visscher and Bowman<sup>2</sup>.

Voge<sup>3</sup> in 1930 using the bromine test for the detection of histidine in urine (Knoop reaction) found that his results closely paralleled those of the Aschheim Zondek reaction. Burt White<sup>4</sup> in 1930 obtained results similar to but less encouraging than those reported by Voge. G H Dodds<sup>5</sup> in 1930, after examining 380 samples of urine, concluded that the test is not specific for pregnancy. Armstrong and Walker<sup>6</sup> in 1932 showed that the Knoop reaction is quite specific for histidine and that only methyl histidine gave a similar reaction. Kapeller Adler<sup>7</sup> in 1933 with a quantitative method determined the amount of urinary histidine excreted normally, in various diseases, and during pregnancy. Seidman<sup>8</sup> in 1935 with a modified Kapeller Adler method, examined 199 samples of urine and concluded that the excretion of histidine is an interesting biochemic phenomenon particularly characteristic of pregnancy but that it is not specific enough to serve as a reliable test for this condition. Foldes<sup>9</sup> in 1936 with his modification of the Kapeller Adler test, reported 44 per cent positive results in a series of urinary specimens from 185 cases of pregnancy. Examination of another series of urinary samples from men and nonpregnant women showed 26.1 per cent positive reactions. He found that the incidence of histidine is greater as the specific gravity of the urine rises, also that histidine is more frequently present in concentrated specimens of urine from pregnant patients than in urinary specimens of similar concentration from men and nonpregnant women. Foldes concluded that such a qualitative test is not of value for the diagnosis of pregnancy.

In 1934 Visscher and Bowman<sup>2</sup> developed a chemical test for pregnancy based on the probable oxidation of sex hormones in urine. They examined 317 specimens of urine from "established cases" with an accuracy of 93 per cent. Menken<sup>10</sup> with this test examined specimens of urine from nonpregnant women known and questionable cases of pregnancy, and concluded that the

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## DETERMINATION OF THE ICTERIC INDEX BY THE ACETONE METHOD\*

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IN THIS report an attempt will be made to show that, by using acetone rather than water or saline as a diluent, the determination of blood icteric index is rendered more accurate. The clinical course of jaundiced patients may be followed more precisely by this method, which may, therefore, frequently serve as a useful alternative procedure to the more complicated quantitative van den Bergh reaction. The latter, moreover, possesses the additional disadvantage of unreliability when the amount of circulating bilirubin is less than 0.5 mg. per cent.<sup>1</sup>

### INTRODUCTION

To meet the demand for a simpler and more satisfactory quantitative method than that of van den Bergh,<sup>2</sup> Meulengracht<sup>3</sup> attempted to measure bilirubin colorimetrically by comparison of the serum with a standard solution (1:10,000) of potassium dichromate. He diluted the serum with normal salt solution, and used, as an arbitrary unit, the number of times the serum had to be diluted to match the standard, the resulting value being designated the icteric index. Meulengracht was of the opinion that bilirubin is in almost all instances the only colored substance to be found in the serum in sufficient concentration to affect the readings significantly.

In 1924, Ernst and Förster<sup>4</sup> reported difficulties arising in the use of the Meulengracht technique. They found that diluting with saline frequently rendered the serum opalescent, causing marked difficulties in matching with the clear bichromate standard. In addition, hemolysis, when present, made accurate comparison impossible. Of greater importance was the fact that considerable hemolysis was not detectable in deeply jaundiced serums, leading to large errors when the icteric indices were determined. They, therefore, sought a diluent which would precipitate proteins without affecting the color of the bilirubin. This would insure a clear filtrate free of hemoglobin, permitting accurate estimation of the icteric index. They added two parts of colorless redistilled acetone to one part of serum; the mixture was filtered and the color of the filtrate was compared with that of a freshly prepared standard solution of potassium dichromate, the serum being diluted further with pure ethyl alcohol until the colors matched. The authors suggested that specimens should be kept from light to prevent oxidation of the bilirubin to biliverdin, etc.

A simple and satisfactory modification of the acetone method of Ernst and Förster, used routinely in this laboratory, has been employed in this study.

\*From the Laboratories of The Mount Sinai Hospital.  
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TABLE I  
RESULTS OBTAINED WITH URINARY SPECIMENS FROM CASES OF PREGNANCY

	SPECIFIC GRAVITY	LESS THAN 1.006	1.006 TO 1.010	1.010 TO 1.015	1.015 TO 1.020	1.020 TO 1.025	1.025 TO 1.030	1.030 TO 1.035	TOTAL
Foldes test	Specimens of urine examined	42	59	56	41	31	11	7	247
	Number positive	0	7	20	29	22	10	7	95
Vischer Bowman reaction	Specimens of urine examined	32	10	26	17	12	5	8	110
	Number positive	7	3	13	10	9	4	7	53

TABLE II  
RESULTS OBTAINED WITH URINARY SPECIMENS FROM MEN AND NONPREGNANT WOMEN

	SPECIFIC GRAVITY	LESS THAN 1.006	1.006 TO 1.010	1.010 TO 1.015	1.015 TO 1.020	1.020 TO 1.025	1.025 TO 1.030	1.030 TO 1.035	TOTAL
Foldes test	Specimens of urine examined	37	27	58	73	60	36	17	308
	Number positive	0	0	0	8	7	6	4	25
Vischer Bowman reaction	Specimens of urine examined	21	8	14	20	11	4	5	83
	Number positive	1	1	2	6	4	2	3	19

# DISCUSSION

From the results obtained it is evident that both tests yield a fairly high percentage of positive results with urinary specimens of high specific gravity from pregnant women, and that the proportion of positive results rises with increase in concentration of the urine. However, specimens of urine from nonpregnant individuals also yield an increasing percentage of positive results as the specific gravity rises. In our study, the Foldes test yielded 13 per cent more false negatives and 14.9 per cent less false positives than did the Vischer Bowman test. A positive Foldes test with urine of specific gravity of 1.012 or less would be strongly indicative of pregnancy, on the other hand pregnancy would be unlikely when specimens of urine with a specific gravity of 1.032 or more give negative reactions (Chart 1). This would also apply to the Vischer Bowman test but to a lesser degree.

method, the curve of averages more nearly approaches a straight line, the large individual variations (as indicated by the mean variation of over 30 units) obviate this advantage.

Values for icteric index above five obtained by the acetone method are regarded as abnormal although occasionally a normal serum will show an icteric index one or two units higher. When a slightly, but definitely, increased icteric index is unconfirmed by the van den Bergh test, the latter is regarded as probably being in error. This has been substantiated repeatedly by clinical observations. It has also been found that hemolysis does not interfere with the accuracy of the acetone method.

#### DISCUSSION

Because of its ease and accuracy, determination of the icteric index by the acetone method provides a convenient means for following the course of patients with jaundice of any type. In cases where differential diagnosis is required, the qualitative van den Bergh is essential. However, when the amount of serum bilirubin is small, determination of the acetone icteric index at frequent intervals provides more accurate information than the quantitative van den Bergh is capable of giving. An important practical advantage is the elimination of hemolysis as a source of error. Hemolysis accounts for some of the more flagrant inconsistencies seen between the quantitative van den Bergh and the icteric index by the saline method. When the amount of circulating bilirubin is high, the quantitative van den Bergh is more reliable than the icteric index.

Certain substances other than bilirubin have been thought to affect the icteric index, notably lipochromes, of which lutein and carotene are the most important. Meulengraecht<sup>3</sup> pointed out that, while lutein occasionally colors the serum in certain animals, it has never been found sufficiently concentrated in human serum to influence the icteric index significantly. Nation and Myers,<sup>4</sup> investigating the question of carotenemia in 1934, found that in a series of 161 cases of which 75 were diabetics, the carotene concentration had a negligible effect (less than one unit) on the icteric index except in rare instances when there could be no question of the diagnosis. Dinitrophenol and atabrin may occasionally become sufficiently concentrated in the blood to cause an elevated icteric index without raising the van den Bergh value.

The icteric index is a valuable procedure if it is remembered that only the intensity of the yellow color in serum is measured.<sup>5</sup> In a given case changes in the color reflect the quantitative changes in the bilirubin. However, in comparing different cases, the color intensity may vary due to other factors, such as qualitative differences in the circulating bile pigments.

#### SUMMARY

The various methods for determining serum bilirubin have been considered. A modification of the acetone method of Ernst and Förster has been presented. The advantages of this method both technically and as an aid in following the progress of jaundice cases have been pointed out.

14. 46° to 48° C. melting point paraffin, fifteen minutes.
15. 50° to 52° C. melting point paraffin, fifteen minutes.
16. Embed in a suitable paraffin.
17. Cool block in water or on ice.
18. Trim paraffin block.
19. Mount on wood fiber block.
20. Cut sections 4 to 6 microns in thickness.
21. Float sections on water (albuminized slide).
22. Flatten by gently heating in Bunsen flame.
23. Drain water, blot and dry in oven.
24. Dissolve paraffin with xylol.
25. Mount in balsam.
26. Examine under oil immersion lens.

**Points To Be Observed In The Silver Impregnation Technic For Leptospirae:**

1. Do not fix in bulk, but cut into strips about 1.5 to 2.0 mm. thick.
2. Do not mix formalin with tap water.
3. Do not fix in alkaline fixatives.
4. Do not wash in tap water at any stage.
5. Do not leave the tissues in the fixative, but store in 70 per cent alcohol.
6. Do not forget to have all required solutions at 50° C. on transfer of tissues.

**TISSUE: Method of Temporarily Preserving Fresh Frozen Sections Stained With Polychrome Methylene Blue, Kernohan, J. W. Am. J. Clin. Path. 6: 185, 1936.**

The method employed is, as soon as the diagnosis is arrived at and before the mounting fluid (glucose or water) has evaporated, to paint around the edge of the coverslip with a clear, quick-drying lacquer (clear "Duco"). This serves to prevent evaporation of the mounting fluid and to seal it. The blue will of course ultimately (after a few days or weeks) go into solution in the mounting medium; but fixed sections have by that time been prepared and examined.

The use of a clear, quick-drying lacquer is not limited to fresh frozen sections, as all microscopic preparations mounted in balsam, or some modification of this mounting medium, can be sealed with the preparation. It is also useful in preserving fat stains by surrounding the cover glass, thus preserving the section indefinitely.

**RENAL FUNCTION, Evaluation of Measures of, in Persons With Arteriosclerotic Bright's Disease, Elliot, A. H., and Nuzum, F. R. Arch. Int. Med. 57: 1152, 1936.**

In 111 cases of arteriosclerotic Bright's disease renal function was measured by the phenolsulphonphthalein test and by the dilution and concentration test of Vollhard. In addition, the urea clearance was determined in 33 patients, creatinine excretion (Major's test) in 34 and the urea nitrogen level of the blood in 67.

Impairment of diluting ability was present in one-fourth of the patients with essential hypertension. The creatinine excretion was low in one-third. Urea clearance, concentrating ability and phenolsulphonphthalein output were each low in one-half of the patients on whom these measurements were made.

Correlation between the results of the tests was vague and little better than could be expected as a chance occurrence. In the individual instance, it was impossible in this study to predict the outcome of any one test on the basis of the result given by another. A possible exception to this was that an increased amount of urea nitrogen in the blood was usually accompanied by a decreased output of phenolsulphonphthalein.

The effect of weakness of the left ventricle on the results of the tests was studied in 21 instances. The phenolsulphonphthalein output was not apparently decreased; the urea clearance was. By the Vollhard technique, a lag in the excretion of water and an impairment of concentrating ability were demonstrated in these patients.

From these above results it may be concluded that the normal urine constituents interfere with the Sumner method for sugar.

Analyses were then conducted on normal urines as controls and the same urines to which a known concentration of glucose had been added. Results are given in Table II.

TABLE II

URINE	MG. AS GLUCOSE IN ORIGINAL	MG. GLUCOSE		MG. AS GLUCOSE RECOVERED	PER CENT RECOVERY
		ADDED	TO BE RECOVERED		
1	0.15	2.00	2.15	1.79	83.3
2	0.58	2.00	2.58	1.79	69.4
3	0.32	2.00	2.32	1.80	77.7
4	0.33	2.00	2.33	1.79	76.9
5	0.67	2.00	2.67	1.95	73.0
6	0.72	2.00	2.72	2.25	82.8
7	0.69	2.00	2.69	1.94	72.2
8	0.19	2.00	2.19	1.80	82.2
9	0.56	2.00	2.56	2.10	82.2
10	0.61	2.00	2.61	1.86	74.2
11	0.79	2.00	2.79	2.17	77.8
12	0.42	2.00	2.42	2.02	83.5

These results show that in actual urinalyses the constituent components of the urine hinder the actual sugar determination as performed by the Sumner method.

## REFERENCES

1. Sumner, J. B.: A More Specific Reagent for the Determination of Sugar in Urine, *J. Biol. Chem.* 65: 393, 1925.
2. Sumner, J. B.: Dinitrosalicylic Acid: A Reagent for the Estimation of Sugar in Normal and Diabetic Urine, *J. Biol. Chem.* 47: 5, 1921.
3. Sumner, J. B.: The Estimation of Sugar in Diabetic Urine, Using Dinitrosalicylic Acid, *J. Biol. Chem.* 62: 287, 1924-25.

due to an absolute or relative increase in the number of small platelets; i.e., those belonging to Group 1. The presence of increased numbers of the larger types of platelets is often associated with intense regenerative activity, abnormal function or hypoplasia of the megakaryocytes in the bone marrow.

Normally functioning platelets are usually normal morphologically. Functionally, the smaller platelets are much more active than the larger types. The small juvenile platelets possess high agglutinating power, and their presence in large numbers constitutes a significant factor in the causation of spontaneous thrombosis in conditions associated with thrombocytosis.

**CIRCULATION TIME, The Use of Calcium Gluconate as a Test for, Goldberg, S. J. Am J M Sc 192: 36, 1936**

The arm to tongue circulation time, a relative index of the rate of blood flow through the lungs, is a useful test of the functional efficiency of the circulation. The velocity with which the blood flows through the lungs is a composite result of the oxygen demands of the body and the capacity of the heart and blood vessels to propel the blood to meet such demands.

A simple method is described which lends itself to general use, and the results in 156 normal and pathologic cases are reported. The calcium gluconate method gives normal readings varying from 10 to 16 seconds, with an average arm to tongue circulation time of 12.5 seconds.

Hyperthyroidism increases the velocity of the blood flow, and cardiac failure markedly slows it. In myxedema and hypothyroidism there is a slowing of the circulation in proportion to the fall in metabolism. The test is helpful in the differentiation of edema of cardiac or renal origin, and may be of assistance in distinguishing between cardiac and bronchial asthma. The calcium gluconate test is approximately accurate, and easy to perform. No untoward effects have been observed, nor has there been any thrombosis or tissue damage in a single instance.

The method follows. The patient reclines, with the arm at the level of the right auricle. After entering the vein, the tourniquet is withdrawn, and the circulation is permitted to return to normal. A stopwatch is held in the left hand, and 3 to 5 c.c. of the 10 per cent solution is injected as rapidly as possible with the other, through an 18 gauge needle. When the larger amount is used, a characteristic response is invariably elicited. The time required for completing the injection is subtracted from the total circulation time, although if a needle of large bore be used, this rarely occupies more than one half second. There has been recently made available a 20 per cent solution of calcium gluconate which permits the use of smaller volumes, and minimizes the factor of injection time. Recent tests have been carried out with 2.5 c.c. of this solution with improved results. There is no pain, thrombosis or slough should the injection be improperly made. The patient announces the onset of the hot sensation in the pharynx by crying out "hot," or some other such signal. The sensation is sudden in onset, and wells up rapidly into the throat very much like a "gust of steam," to use the expression of one patient. It is next felt in the face, and then successively in the anterior chest, perineum, the hands, and finally the feet. These effects depend upon the arrival of the solution in the respective peripheral arterial beds. The onset of the sensation in the throat and tongue is most intense, as the substance is here present in greatest concentration.

After the sensation has entirely subsided, usually within one or two minutes, the reading may be repeated, without removing the needle from the vein. The second reading usually checks closely with the first, indicating that the first injection of the material has no great effect on the circulation itself. No discomfort is experienced by the patient as a result of the rapid injection, even in those with advanced heart failure.

test is an aid to the practicing physician in the diagnosis of pregnancy. Dolff<sup>11</sup> used this test and obtained 96.08 per cent positive results in late pregnancies, 94.45 per cent in early pregnancies, and 81.82 per cent in ectopic pregnancies. He found that concentrated specimens of urine from men and from nonpregnant women, which contain reducing products of metabolism, often yield a positive reaction, and he suggested that more material be studied before conclusions are drawn regarding the value of the test.

We first made a comparative study of the tests as outlined by Voge, Kapeller-Adler, and Földes. From the results obtained, we concluded that the Voge test is the least efficient, giving the smallest number of correct positive results, and that the Kapeller-Adler method is less satisfactory than the procedure suggested by Földes.

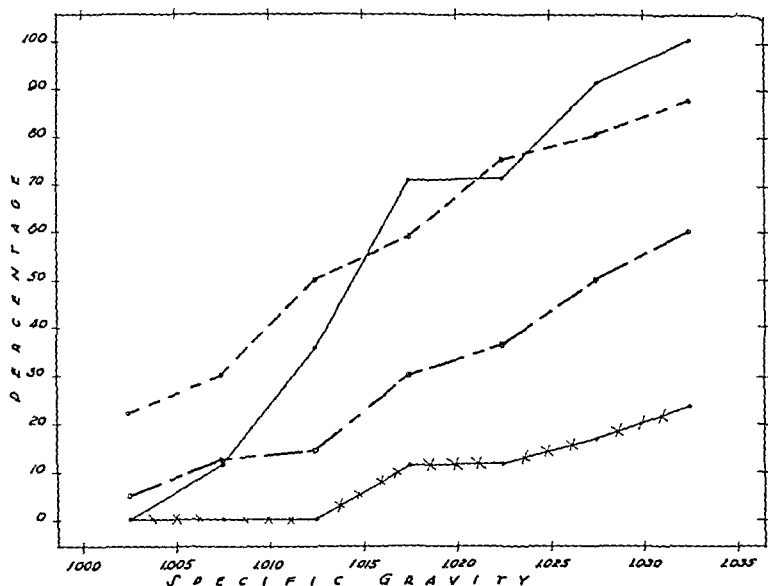


Chart 1—Results of the Földes and Visscher-Bowman tests in terms of the percentage of positive results. ————— Földes test, and ——— Visscher-Bowman test, with specimens of urine from cases of pregnancy. —x—x—x— Földes test, and —+—+—+— Visscher-Bowman test, with specimens of urine from nonpregnant individuals

The method of Földes was followed except that we omitted the use of potassium iodide starch solution as an indicator for the exact amount of bromine reagent to be used, because we found that this does not add materially to the sensitiveness of the test.

Following our preliminary work with the bromine tests a study was made to determine the comparative value of the Földes and Visscher-Bowman tests. The 748 samples of urine which we tested were received by the hospital laboratory for routine examination.

Table I shows the results of the Földes and Visscher-Bowman tests obtained with specimens of urine of varying specific gravity from cases of pregnancy of at least three months' duration, and Table II presents a similar study of urinary samples from men and nonpregnant women. Chart 1 records the results in terms of the percentage of correct and of false positive reactions.

# Venereal Disease Information

A monthly publication prepared by the U. S. Public Health Service for distribution among the medical profession throughout the United States. It measures approximately 6 by 9 inches and ranges in size from 25 to 75 pages.

It is the purpose of the Public Health Service in issuing this publication to provide in condensed form a monthly summary of the scientific developments in the diagnosis, treatment, and control of syphilis and gonorrhea. More than three hundred American and foreign journals are reviewed for this work. Abstracts are made of articles describing laboratory, pathologic, and clinical work in the field of venereal diseases.

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In seltener Geschwindigkeit folgt schon die 3. Auflage des erst vor wenigen Jahren neuerschienenen Lehrbuches und zeigt damit, dass Herausgeber und Verlag den richtigen Weg gewählt haben, um in knapper Form über alles Wissenswerte aus dem grossen Gebiet der allgemeinen und speziellen Pathologie innerer Krankheiten zu unterrichten. Von den Fragen allgemeiner Pathogenese und Pathophysiologie bis zu den konkretesten Einzelheiten der Therapie ist nichts vergessen, so dass das Werk—angesichts des Mangels eines zeitgemässen Handbuches—geradezu als Extrakt der heutigen deutschen internen Klinik bezeichnet werden kann. . . Einige Kapitel wurden neu geschaffen . . . Das Werk wird weiter seine führende Stellung behaupten und ausbauen.

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It is evident that specimens of urine with a high specific gravity both pregnant and nonpregnant individuals often contain a substance yields a positive reaction with the Földes and Visscher-Bowman tests. mens of urine from pregnant women contain this substance more frequently, however, than do those of nonpregnant individuals.

#### CONCLUSIONS

1. Studies of the Voge, Kapeller-Adler, and Földes tests showed the named test to be the most satisfactory.

2. Samples of urine with high specific gravities from normal individuals and from pathologic states unrelated to pregnancy often yield positive Visscher-Bowman reactions.

3. The Földes test gives more false negative and less false positive reactions than does the Visscher-Bowman test.

4. Our results show that neither the Földes nor the Visscher-Bowman is satisfactory for the diagnosis of pregnancy.

#### REFERENCES

1. Editorial: Urinary Histidine in the Diagnosis of Pregnancy, *J. A. M. A.* 106: 1936.
2. Visscher, J. P., and Bowman, D. E.: Chemical Determination of Pregnancy, *Proc. Roy. Soc. Med.* 31: 460, 1934.
3. Voge, C. I. B.: A Simple Chemical Test for Pregnancy, *Brit. M. J.* 2: 829, 1929.
4. Burt-White, H.: Discussion of paper by J. H. Hannan, entitled "The Detection of the Presence of the Hormone of the Anterior Pituitary Body in the Urine as an Aid in the Diagnosis of Pregnancy," *Proc. Roy. Soc. Med.* 23: 639, 1930.
5. Dodds, G. H.: Value of the Bromine Test for Diagnosis of Pregnancy, *Brit. M. J.* 4: 948, 1930.
6. Armstrong, A. R., and Walker, E.: The Bromine Reaction of Pregnancy Urine, *Brit. M. J.* 26: 143, 1932.
7. Kapeller-Adler, R.: Über eine neue Methode zur quantitativen Histidinbestimmung über deren Anwendbarkeit zur Untersuchung von biologischen Flüssigkeiten insbesondere von Gravidenharnen, *Biochem. Ztschr.* 264: 131, 1933.
8. Seidman, T. R.: The Determination of Urinary Histidine as a Chemical Test for Pregnancy, *Am. J. Obst. & Gynec.* 29: 451, 1935.
9. Földes, F.: Das Vorkommen des Histidins im Menschlichen Urin, *Biochem. Ztschr.* 264: 199, 1933.
10. Menken, J. G.: Chemical Pregnancy Reaction of Visscher & Bowman, *Nederl. tijdschr. v. geneesk.* 79: 979, 1935. Abstr. *Deutsche med. Wchnschr.* 60: 1837, 1934.
11. Dölff, C.: Bericht über einige Nachuntersuchungen der Chemischen Schwangerschaftsreaktion von Visscher & Bowman, *Zentralbl. f. Gynäk.* 59: 2901, 1935.

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\*J. A. M. A. 108: 1054, March 27, 1937.

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The significance of nocturia in patients with weakness of the left side of the body, traceable to a delay in the diuretic response to fluids consumed in the latter part of the day, is discussed. When shown without obvious cause by such patients nocturnal polyuria is primarily of cardiac origin and its presence may be of value in the diagnosis of impending failure of the left ventricle.

It is difficult to interpret the results of tests of renal function in persons with arteriosclerotic renal disease in terms of the actual amount of healthy renal parenchyma. The value of the available tests is in aid to prognosis is disappointing.

# PLATELET COUNT, The Differential Of: I Arch Int Med 57 1167, 1936

The preserving fluid employed in this investigation for both total and differential platelet counting has the following composition

Sodium met uphosphate	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

Under optimal conditions the platelets in this solution, when observed with the oil immersion lens, appear as free floating isolated clear highly refractive bodies with numerous spinelike processes projecting from the periphery. Most of the platelets are fairly round or ovoid, irregularly shaped thrombocytes especially elongated forms considered by Ayman to be the common physiologic type are seen rather infrequently in these preparations. If it is desirable to stain the platelets the following solution may be used

Sodium met uphosphate	1.00
Sodium chloride	0.40
Dextrose	0.10
Sodium bicarbonate	0.10
Brilliant cresyl blue	0.15
Distilled water	100.0

The palmar surface of the fingertip is punctured with an autowrite lancet after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether. The first drop or two of blood is discarded. A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin and the hand is quickly turned over so that the palmar surface is directed downward. After a sufficiently large drop of blood has escaped into the drop of diluent, the mixture is applied to the surface of a small quantity (3 or 4 drops) of diluting fluid contained in a paraffin cup. The mixture is gently stirred and then transferred by means of a paraffin coated applicator to a glass slide, usually three preparations can be obtained, as the quantity of fluid in the cup yields 3 large drops. A cover slip is placed over each drop, and after the preparations have been allowed to stand for from ten to fifteen minutes a relative thrombocyte erythrocyte count is made the oil immersion lens being used. Sealing the edges of the preparations with liquid petroleum will prevent air currents in them. An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined. With this method the average number of platelets per cubic millimeter in normal adults is about 500,000.

The normal platelets can be differentiated into four groups according to size. Group 1, consisting of platelets 1.8 microns in diameter, Group 2, consisting of platelets 2.5 microns in diameter, Group 3, consisting of platelets 3.6 microns or more in diameter, and Group 4, consisting of irregular shaped platelets. In normal persons 18.6 per cent of all the circulating platelets belong to Group 1, 63.3 per cent to Group 2, 17.4 per cent to Group 3 and 0.7 per cent to Group 4.

In conditions associated with thrombocytosis and at times in those associated with thrombopenia, the deviation from the normal in the differential platelet formula is usually

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**CHLORIDES, Urinary Determination of** Fantus B J A M A 107 14 1936

The following test is described in a paper on the postoperative use of fluid (fluid post operatively)

Place 10 drops of urine in a test tube. Add 1 drop of a 1 to 5 potassium chromate solution. The fluid will now assume a somewhat distinctly yellow color. Add drop by drop with the same dropper or one of the same caliber a 2.9 per cent silver nitrate solution until a permanent and distinct color change to red brown occurs (resulting from the formation of silver chromate). The number of drops required to produce the change of color expresses in grams the content of chloride per liter of urine.

**HYPERCALCEMIA Induced Its Possible Therapeutic Relation to Thrombocytopenic Purpura**, Lowenburg H Sr and Ginsburg T M J A M A 106 21, 1979

A second case of acute hypercalcemia produced by intentional overdosage with parathyroid extract occurred in a boy with thrombocytopenic purpura.

Toxic symptoms occurring in man are similar to those reported by many workers as occurring in animals with experimental hypercalcemia.

The earliest symptom is vomiting. This is followed shortly by weakness, apathy, and lethargy.

Both patients on whom hypercalcemia was established presented definite objective changes in the blood (bleeding time, clotting time, clot retraction, platelet count) as well as clinical cure.

A cause and effect relationship between the hypercalcemia and the apparent cures is suggested although there appear to be no sound theoretical grounds for such a conclusion.

Calcium gluconate was used to protect the bones from the withdrawal of calcium from them into the blood.

**HODGKIN'S DISEASE The 'Gordon Test' for** Goldstein J D Am J M Sc 191 77, 1936

Biopsy material from 7 to 9 cases of Hodgkin's disease produced an encephalitic syndrome (a positive "Gordon test") when inoculated intracerebrally into rabbits.

Twenty control lymph nodes, including 5 tuberculous nodes and 2 nodes from infectious mononucleosis, were negative.

There were no false positive tests. Two of 9 nodes from established cases of Hodgkin's disease failed to give a positive test.

**TYPHOID Production and Persistence of H & O Agglutinins and Complement Fixing Bodies in Persons Inoculated With Typhoid Endotoxoid Vaccine** Grasset, E, and Lewin W Brit J Exper Path 17 179 1936

The sera of individuals inoculated with typhoid endotoxoid vaccine produced by the South African Institute for Medical Research showed the presence of H and O agglutinins in the large majority of cases in high titer. A concentrated antigen produced a higher O agglutinin response than this regular product.

The titer for H agglutinins shortly after inoculation is much higher than for O, but the titer of the latter remains at a more constant level, the H titer falling rapidly.

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**REFERENCE**—Marshall, *Proc. Soc. Exper. Biol. & Med.* 36, 422 (1937)

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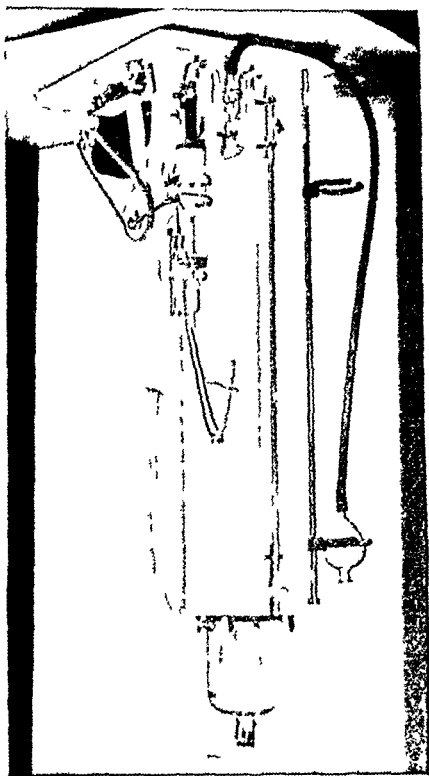
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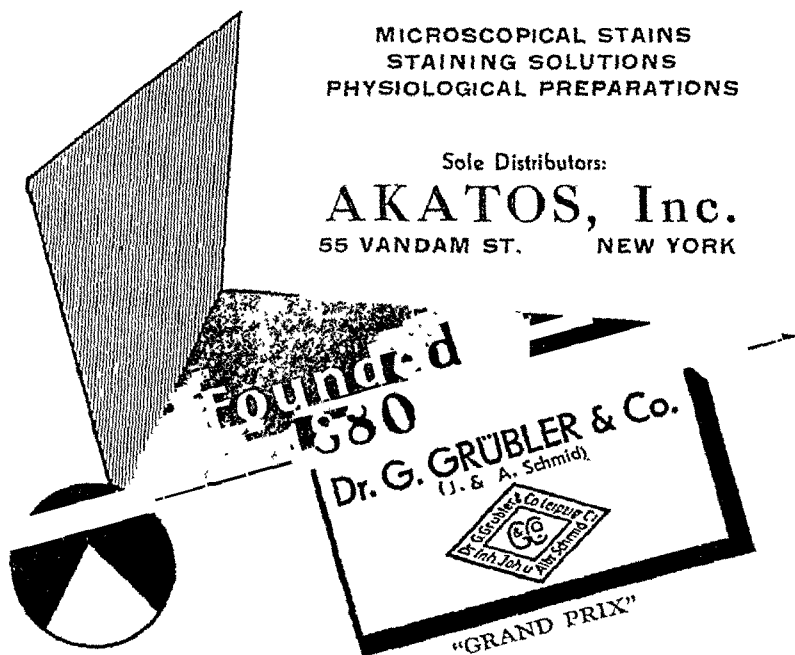
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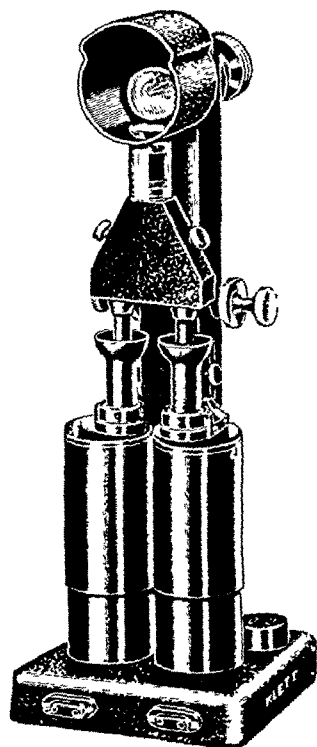
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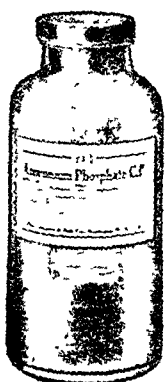
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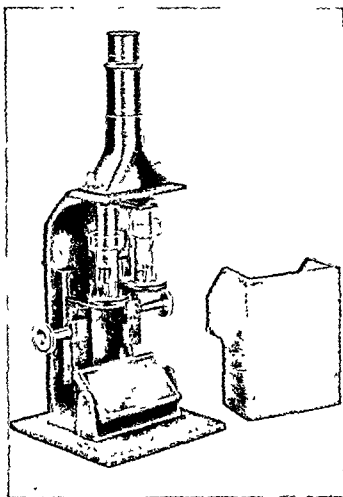
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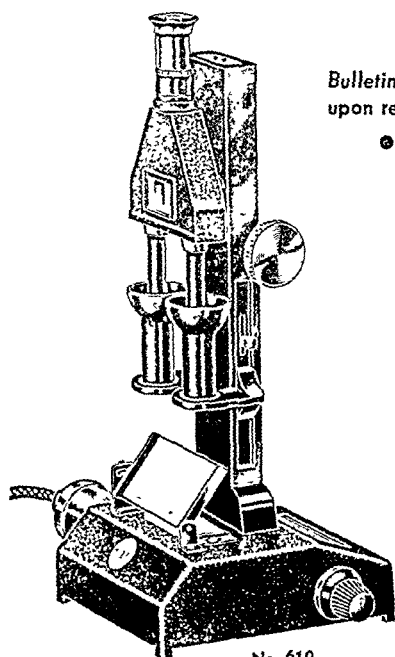
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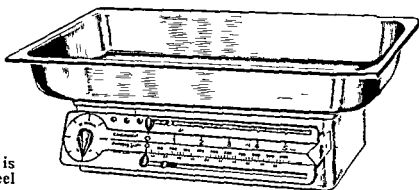
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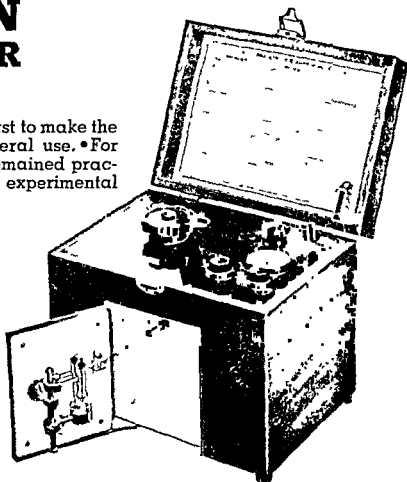
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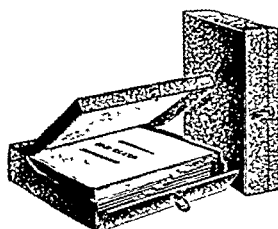
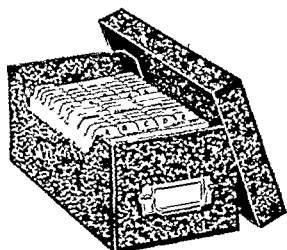
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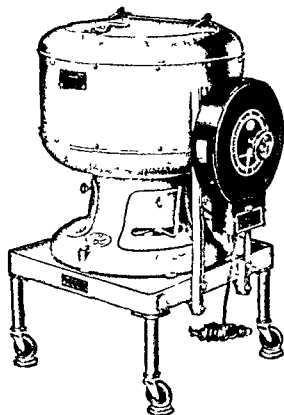
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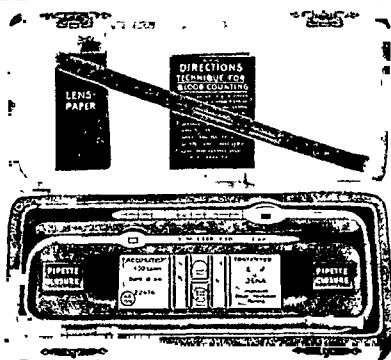
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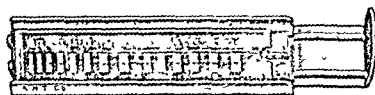
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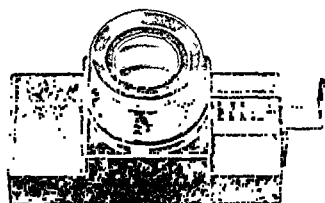
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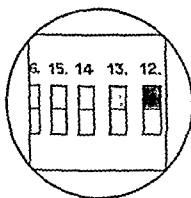
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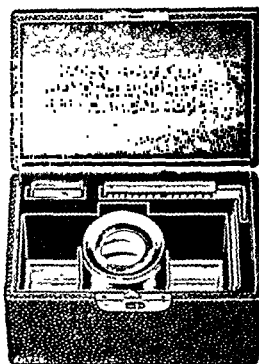
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4.	11.5	11.5	11.2	10.9
5.	13.0	13.0	13.0	12.8
6.	14.0	14.0	14.3	14.0
7.	15.5	15.5	15.7	15.4
Mean	11.5	11.6	11.5	11.3

\*Russell L. Haden, *Jour. of Lab. and Clin. Med.*, Vol. 20, No. 7 (April, 1935), p. 762.

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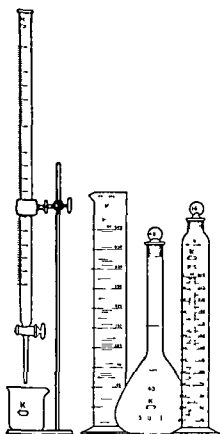
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estimation. He found that since creatine was extracted along with the guanidine bases, and gave a color with the color reagent, it was necessary to apply a correction for the color due to creatine. Using this method, Major and Weber<sup>11</sup> again noted an increase in the blood guanidine values in hypertension and in terminal nephritis. The increased blood guanidine values in hypertension were very irregular, did not follow the blood pressure, and were usually accompanied by an elevation of the blood uric acid and nonprotein nitrogen. A few cases of uremia showed values as high as 2.3 mg. per 100 c.c. of blood. Major and Weber also noted that the injection of sufficient guanidine into the blood to double the blood guanidine concentration, caused a marked increase in the blood pressure.

Nakazawa and Abe,<sup>12</sup> in 1928, found that injections of methyl guanidine into the brain and spinal cord gave results similar to intravenous injections. Major and Weber<sup>11</sup> further demonstrated that injections of methyl guanidine into adrenalectomized animals gave the same rise in blood pressure as with normal animals, showing that the effect of guanidine is not due to a stimulation of the adrenals.

In another paper, Major<sup>14</sup> reported a study of the guanidine of additional cases of hypertension and nitrogen retention, using the charcoal method. He again observed a definite hyperguanidinemia in all cases of nitrogen retention. The increase in blood guanidine appeared to somewhat parallel the degree of azotemia, although he did not mention this point. Over 60 per cent of his cases of hypertension showed some increase in the blood guanidine concentration. It is significant to note that most of the cases of hypertension with guanidinemia also showed some elevation of the blood uric acid or nonprotein nitrogen, indicating a tendency for renal insufficiency. In an address in 1932, the same author<sup>15</sup> stated that he had confirmed these findings with 400 additional cases.

In 1930, Pfflner and Myers<sup>16</sup> described a modification of Major and Weber's charcoal method, whereby the creatine correction is eliminated by the use of the autoclave. They also showed a possible hyperguanidinemia in a few cases of hypertension, but these cases also exhibited a tendency for nitrogen retention. The same year Remond and Colombies<sup>17</sup> reported to have found no relationship between guanidine and the nonprotein fraction of the blood. They found a definite hyperguanidinemia in some of their cases of hypertension, and further observed a lowering of the blood guanidine concentration in these cases by administering parathormone. De Wesselow and Griffith<sup>18</sup> and also Turries<sup>19</sup> reported a similar relationship between hypertension, nitrogen retention, and blood guanidine.

Pekelis and Parenti<sup>20-23</sup> in several papers (their analytical data are so erratic as to really confuse the issue) appear to have shown a hyperguanidinemia in most cases of nitrogen retention, although the increase is not proportional to that of the other nitrogenous constituents. Most of their cases of essential hypertension showed normal values for blood guanidine. Zappacosta<sup>24</sup> with a new method reports similar findings.

Marcologo and coworkers<sup>25, 26</sup> appear to have found essentially normal blood guanidine values in all their cases of hypertension, but observed a definite

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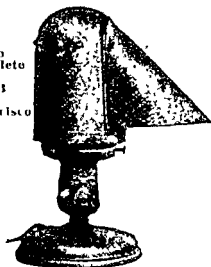
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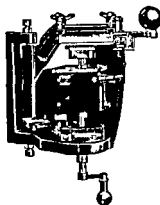


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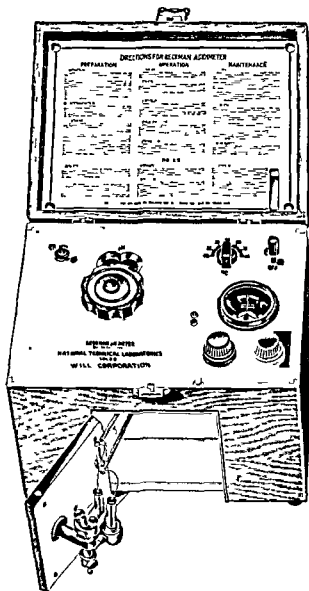
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In Table I the guanidine values are tabulated before and after correcting for the conversion of creatinine into guanidines by the charcoal. The corrections are small when compared with the absolute values for the blood guanidine.

TABLE I  
BLOOD GUANIDINE WITH NITROGEN RETENTION  
(Figures in mg. per 100 c.c.)

CASE	AGE AND SEX	UREA NITROGEN	CREATININE	SUGAR	GUANIDINE DE-TERMINED	GUANIDINE CORRECTED	BLOOD PRESSURE mm. Hg	CLIN. DIAGNOSIS AND REMARKS
1	32 M	18	2	95	0.31	0.28		Acute hepatitis
2	46 M	21		26	0.35	0.32		Adenoma of the pancreas
3	59 M	35	2.3		0.35	0.32	110/74	Gen. paresis
4	29 F	15		85	0.36	0.33	200/140	Chr. glomerulonephritis
5	48 M	34	2		0.37	0.35	106/72	Gen. paresis
6	43 M	17	2		0.37	0.35	120/85	Portal cirrhosis
7	43 M	21		93	0.41	0.38	125/85	Portal cirrhosis
8	53 M	26		152	0.42	0.39	236/140	Nephrosclerosis
9	58 M	40	4	93	0.44	0.39	240/130	Nephrosclerosis
10	68 M	21	2.2		0.43	0.40	100/50	Portal cirrhosis
11	40 M	27	2.5		0.48	0.44	250/168	Nephrosclerosis
12	89 M	17		95	0.49	0.45	118/92	Prostatic obstruction (Urea had been high)
13	56 M	22			0.54	0.51	198/130	Nephrosclerosis
14	56 F	49	6	174	0.59	0.51	150/90	Diabetes and Chr. nephritis
15	55 M	75	7		0.63	0.53	260/138	Chronic nephritis
16	36 F	37	3	102	0.60	0.56	190/140	Nephrosclerosis
17	31 F	76	7	449	0.65	0.56	110/78	Diabetes and Chr. nephritis
18	54 M	95	8		0.70	0.59	165/95	Chronic nephritis
19	17 F	31	2	170	0.63	0.60	250/190	Nephrosclerosis
20	64 M	71	7		0.83	0.73	160/80	Prostatic obst.
21	50 M	49	6		0.86	0.78	152/90	Polycystic kidneys
22	16 F	120	8		0.90	0.79	210/150	Glomerulonephritis
23	69 M	92	7	170	0.90	0.80	235/150	Nephrosclerosis
24	36 F	60	8	100	0.92	0.81	195/148	Nephrosclerosis
25	43 M	88	4		1.00	0.94	182/74	Glomerulonephritis
26	24 F	84	13	108	1.15	0.98	120/70	Glomerulonephritis
27	31 M	160	14		1.20	1.00	220/160	Nephrosclerosis
28	43 M	141	7	142	1.11	1.01	128/103	Renal T.B.
29	50 M	56	8		1.13	1.02	150/90	Polycystic kidneys
30	33 F	35	5		1.10	1.03		Eclampsia and nephritis
31	32 M	159	8	80	1.14	1.03	100/70	Acute hepatitis with jaundice
32	F	42	7		1.17	1.07		Eclampsia and nephritis
33	36 F	69	11		1.25	1.10	190/130	Chronic nephritis
34	34 F	97	16		1.35	1.13	220/140	Chronic nephritis
35	24 F	110	16	118	1.37	1.15	120/60	Chronic nephritis
36	37 F	114	12		1.35	1.17		HgCl <sub>2</sub> poisoning
37	79 M	100	20	140	1.40	1.12	186/138	Chronic nephritis
38	31 M	207	14	134	1.44	1.24	220/168	Glomerulonephritis
39	32 M	199	15		1.49	1.28		Glomerulonephritis
40	24 F	181	19		1.59	1.32	140/75	Glomerulonephritis
41	35 F	150	16	128	1.57	1.35	260/160	Nephrosclerosis
42	54 F	150	17		1.66	1.42	210/140	Chronic nephritis
43	26 M	186	16		1.65	1.43	130/70	Glomerulonephritis
44	42 M	182	15		1.71	1.50	220/130	Chronic nephritis
45	72 M	210	19		1.88	1.61	150/80	Adenoma of prostate
46	59 F	156	12		1.80	1.63	240/140	Nephrosclerosis
47	30 F	191	23	215	2.00	1.68	210/140	Chronic nephritis
48	37 M	257	16	119	2.50	2.28	80/28	Chronic nephritis
49	48 M	311	25	111	3.00	2.65	210/150	Chronic nephritis

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below normal. Other high values with normal blood pressures can be seen in Cases 31, 35, 40, 43, and 44 (see also Case 3, Table II). However, it appears that cases with high blood pressures are accompanied by a higher guanidine value, than those with a normal blood pressure, where the degree of azotemia is similar.

#### BLOOD GUANIDINE IN HYPERTENSION WITHOUT NITROGEN RETENTION

In Table III are presented guanidine determinations on the blood of 12 cases showing hypertension without nitrogen retention (if one may judge by figures for the blood urea). In these cases, the systolic blood pressures range from 180 to 275 mm. of mercury. The first 11 cases show guanidine values within the normal range, the average value being about 0.23 mg. per 100 c.c. of whole blood. This is essentially the same average figure as that given for

TABLE III  
HYPERTENSION UNCOMPLICATED WITH NITROGEN RETENTION  
(Figures in mg. per 100 c.c.)

CASE	AGE AND SEX	BLOOD GUANIDINE	UREA NITROGEN	BLOOD PRESSURE mm. Hg	DIAGNOSIS
1	54 M	0.25	8	198/130	Essential hypertension
2	61 M	0.26	11	200/120	Essential hypertension
3	67 M	0.19	11	196/127	Essential hypertension
4	54 M	0.28	9	180/130	Essential hypertension
5	51 M	0.24	15	186/120	Essential hypertension
6	70 M	0.21	10	215/140	Cerebral arteriosclerosis with hypertension
7	55 M	0.23	11	200/150	Hypert. heart disease
8	53 M	0.28	14	190/120	Hypert. heart disease
		0.24	12	210/140	Hypert. heart disease with arteriosclerosis
9	33 F				
10	33 F	0.19	12	178/100	Essential hypertension and pregnancy
11	35 F	0.19	13	190/135	Essential hypertension
12	55 M	0.56	14	275/170	Hypertensive heart disease, arteriosclerosis, and hemiplegia

normal individuals, and indicates no hyperguanidinemia whatever. On the other hand, Case 12 shows a distinct elevation in the blood guanidine concentration. This case undoubtedly falls in the category of Böhm and Schlapp's cases<sup>28</sup> of nephritis that exhibited hyperguanidinemia without azotemia, as autopsy revealed a marked sclerosis of the arterioles of the brain and kidney. It would seem that in this case, the increase in blood guanidine was connected with the renal pathology, the hyperguanidinemia appearing before any other signs of azotemia.

#### CONCLUSIONS

1. Blood guanidine determinations have been carried out on the blood of a series of patients exhibiting nitrogen retention, the renal insufficiency arising from a variety of kidney disorders.

2. In these cases, blood guanidine concentration was found to follow, in general, the degree of azotemia, as measured by the values for blood urea and creatinine. Although the guanidine concentrations did not strictly parallel

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## CLINICAL AND EXPERIMENTAL

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### GUANIDINE LIKE SUBSTANCES IN THE BLOOD\*

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#### II BLOOD GUANIDINE IN NITROGEN RETENTION AND HYPERTENSION

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JEROME E. ANDERSON, PH.D., M.D., MORGANTOWN, W. VA., CHARLES R. LINEGAR,  
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MAJOR,<sup>1</sup> in 1925, using Sharpe's picric acid method, reported a decreased guanidine excretion in patients with nephritis and hypertension. He also noted an increase in the blood pressure when methyl guanidine was injected into the blood of dogs, and found that the rise was due to a constriction of the arterioles. In another paper,<sup>2</sup> he reported an increase in the guanidine excretion in patients with decreasing blood pressure. He further showed<sup>3</sup> that guanidine compounds produced no rise in the blood pressure, if they were injected slowly, and in 1926, he demonstrated<sup>4</sup> that hepatic extracts lower the hypertension produced by guanidine injection, by dilating the capillaries. However, Greenwald<sup>5, 6</sup> and White,<sup>7</sup> in the same year, showed quite conclusively that Sharpe's picric acid method is valueless for the determination of guanidine bases in the urine. Piffner and Myers,<sup>8</sup> in 1926, using a method whereby the guanidine bases are determined directly on the Folin Wu blood filtrate by the use of Marston's color reagent, showed an increase in the blood guanidine of patients with hypertension and nephritis. At about the same time Major and Weber,<sup>9</sup> using a similar method with a modified color reagent found a similar increase in the blood guanidine of patients with hypertension and uremia.

Later Weber<sup>10</sup> described a new method in which the guanidine bases were extracted from the blood filtrate by blood charcoal previous to colorimetric

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\*From the Department of Biochemistry, School of Medicine, Western Reserve University.  
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24. Zappacosta, M.: A New Method for Determining Methyl Derivatives of Guanidine in the Blood. Preliminary Observations in Essential and Renal Hypertension, *Bull. soc. ital. biol. sper.* 10: 705, 1935.
25. Marcolongo, F.: La guanidina nella fisiopatologia e nella clinica del nefropatie, *Minerva med.* 2: 501, 1933.
26. Marcolongo, F., and Bernabo-Silorata, A.: Ricerche cliniche sulla guanidina nelle ipertension arteriose, *Clin. med. ital.* 65: 23, 1924.
27. Kleeberg, P., and Schlapp, W.: Über die Auffindung von urämieerzeugenden Stoffen, *Ztschr. f. physiol. Chem.* 188: 81, 1930.
28. Böhn, H., and Schlapp, W.: Untersuchungen zum Mechanismus des blässen Hochdrucks: V. Der Guanidingehalt des Bluts beim blässen und roten Hochdruck, *Zentralbl. f. inn. Med. No. 18a* 53: 571, 1932.
29. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in the Blood. I. Colorimetric Estimation and Normal Values, *J. LAB. & CLIN. MED.* 22: 1147, 1937.

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## A COMPARATIVE STUDY OF ORAL AND SUBCUTANEOUS VACCINATION AGAINST TYPHOID FEVER BASED ON AGGLUTINATION TITRATIONS\*

H. D. MOOR, M.S., M.D., AND IDA LUCILLE BROWN, M.S., M.T.,  
OKLAHOMA CITY, OKLA.

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IN THE September, 1935, number of the *Oklahoma State Medical Journal*, we published a preliminary report on the oral administration of typhoid vaccine. In this paper, we reviewed the literature dealing with this problem, especially the work of Lloyd Arnold,<sup>1</sup> Finder and Simons,<sup>6</sup> Besredka,<sup>2</sup> Garbat,<sup>7</sup> Gauthier,<sup>8</sup> Burke and others,<sup>3</sup> Cluver,<sup>4</sup> Pirie and Orenstein,<sup>11</sup> Hoffstadt and others,<sup>9</sup> Pijper and Dau,<sup>10</sup> Simons,<sup>12</sup> and Tuft and others.<sup>13</sup> We found by this review of the literature that most of the laboratory experiments carried out in connection with the study of oral typhoid vaccine have been accomplished by using animal subjects. It is true that a great number of persons in Africa, South America, and Europe have been given typhoid vaccine orally with a marked reduction in the incidence in those places as reported by the above investigators, but a comparative study of the oral and subcutaneous typhoid vaccine was not made. No comparative study has been reported to date as far as we have been able to learn except those in which animals were used as experimental subjects.

The results we obtained with the ten individuals reported in our preliminary study stimulated us to make the investigation we are now reporting. We have during the past two years carried out a comparative study of this problem, using 187 human subjects. The results of our investigations can best be shown in Tables I to VI and the discussion of each.

In this experiment, as is shown by Table I, we used 10 persons and gave them typhoid vaccine subcutaneously, 3 doses one week apart, 0.5 c.c. the first dose and 1 whole c.c. for each of the other two. This vaccine was the so-called triple typhoid vaccine composed of 1,000 million typhoid bacilli per

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\*From the Department of Bacteriology, University of Oklahoma School of Medicine. Received for publication, January 8, 1937.

hyperguanidnemia in cases of kidney disease with nitrogen retention (somewhat paralleling the degree of azotemia) Schlapp and coworkers<sup>27-28</sup> published two excellent papers based upon their experimental work. They observed that essential hypertension without any signs of kidney involvement, always shows normal values, but that chronic kidney disease with hypertension is always accompanied by a definite hyperguanidnemia, regardless of whether nitrogen retention is present or not. They found no direct relationship between the degree of azotemia and hyperguanidnemia, although in general higher values for urea and creatinine were accompanied by a similar increase in guanidine. They criticized (and apparently justly) previous investigators for not properly classifying their cases, giving this as the explanation for their observing hyperguanidnemia in only *part* of their cases of hypertension without nitrogen retention.

Summing up the results of previous work, it would seem that the level of blood guanidine is not related to hypertension *per se*, but is closely related to hypertensive nephropathies, especially when azotemia is present.

#### EXPERIMENTAL

The method employed is a modification of the charcoal extraction procedure, in which the necessity for a correction for the color given by creatine is eliminated. Although creatine and creatinine are both slightly converted into guanidine bases by charcoal methods, the conversion is quite constant for normal blood. In our work, only the correction for the conversion of creatinine was applied. Normal values with this method average about 0.24 mg per 100 c.c., with a range of 0.18 to 0.28 mg. (See Paper I of this series<sup>29</sup>).

Table I gives the results of guanidine determinations on the blood of patients with nitrogen retention, the cases being arranged in order of increasing guanidine values. The diagnoses of these patients include chronic glomerulonephritis, nephrosclerosis, polycystic kidney, mercuric chloride poisoning, prostatic obstruction, and other conditions with renal insufficiency. Even in a tabulation of this variety of diseases the blood guanidine concentration, in general, parallels the degree of nitrogen retention, as shown by the values for blood urea and creatinine.

Table II, presenting the results of the cases where more than one determination was possible, brings out the parallelism with the nitrogen retention more strikingly. This table also indicates that the rise in the blood guanidine is not proportional to the increase in either the blood urea or creatinine alone. In Case 4, the urea nitrogen increased from 160 to 207 mg per 100 c.c. while the creatinine remained constant. At the same time, the blood guanidine increased from 1.00 to 1.24 mg. In Case 5, the urea nitrogen remained nearly stationary (increased only from 60 to 69 mg), while the creatinine increased from 8 to 11 mg. At the same time, the blood guanidine concentration increased from 0.81 to 1.10 mg per 100 c.c. In both of these cases, the blood pressures were practically constant throughout. In Cases 1, 2, 3, and 6, the guanidine is shown to vary roughly with the changes in the concentration of urea and creatinine, the blood pressures again being practically constant.

TABLE II  
 ORAL ADMINISTRATION OF TYPHOID VACCINE  
 Titration of agglutinin antibodies at weekly intervals. Vaccine started Feb. 21, 1936.

PATIENT	HISTORY OF PREVIOUS VACCINATION	AGG. TITER FEB. 23	AGG. TITER MARCH 2	AGG. TITER MARCH 9	AGG. TITER MARCH 16	AGG. TITER MARCH 23	AGG. TITER MARCH 30
1	9 months ago oral	1-40	1-80	1-160	1-320	1-320	1-160
2	6 months ago	1-40	1-160	1-320	1-640	1-640	1-320
3	7 months ago	1-80	1-80	1-160	Individual ill, aggl. not taken	1-640	1-320
4	3½ yr. ago	No aggl.	1-80	1-80	1-320	1-320	1-160
5	2 yr.; typhoid 19 yr. ago	1-40	1-80	1-160	1-320	1-640	1-320
6	6 yr. ago	No aggl.	1-40	1-80	1-160	1-320	1-160
7	8 mo. ago	No aggl.	1-80	1-160	1-320	1-640	1-320
8	Oral 10 mo. ago	No aggl.	1-40	1-160	1-320	1-640	1-320
9	6 mo. ago	No aggl.	No aggl.	1-160	1-320	1-640	1-320
10	15 years	1-160	1-320	1-640	1-640	1-1280	1-640
Average aggl. titer		1-36	1-96	1-208	1-352	1-604	1-304

Even in Case 49 (an extremely severe case of nitrogen retention) this correction only amounts to about 0.35 mg of methyl guanidine, as compared to the determined value of 3.00 mg (corrected value = 2.65 mg). A correction for the

TABLE II  
BLOOD GUANIDINE IN INDIVIDUAL CASES OF NITROGEN RETENTION  
(Figures in mg per 100 cc)

CASE	AGE AND SEX	DATE 1931	BLOOD GUANIDINE	UREA NITROGEN	CREATININE	BLOOD PRESSURE mm Hg	DIAGNOSIS
1	50 M	5/9 5/22	0.78 1.02	49 56	6 8	152/90 150/90	Polycystic kidney
2	33 F	6/12 6/15 6/22	1.07 1.03 0.95	42 35 41	7 5 4		Eclampsia and uremia
3	24 F	11/9 11/10 11/20	0.98 1.15 1.32	84 110 181	13 16 19	120/70 120/60 140/75	Chronic glomerulo nephritis
4	31 M	11/11 11/24	1.00 1.24	160 207	14 14	220/160 220/168	Nephrosclerosis
5	36 F	5/30 6/1	0.81 1.10	60 69	8 11	195/148 190/130	Chronic nephritis
6	43 M	10/29 1/27	0.55 0.38	17 21	2 2	120/85 125/80	Alcohol cirrhosis

creatinine conversion should also be applied, but creatinine determinations were not possible due to the lack of sufficient blood. This correction would be smaller than that for creatinine, and would not influence the general value of the results presented.

It can also be seen from the values given in Table I, that in every case where the urea nitrogen was above 15 mg per 100 cc, the blood guanidine was above the normal range. In fact, it would seem that the guanidine in some cases is retained before the urea. In decreasing kidney function, it is known that uric acid is increased first, urea next, and finally creatinine (and creatine). It may be that a hyperguanidinemias is found in some cases (especially those with high blood pressure), before a definite increase in blood urea. In fact, this is entirely in agreement with the work of Schlapp and coworkers<sup>28</sup>. Certainly, in our work an increase in the blood guanidine concentration was observed in every case when the blood urea nitrogen was above 15 mg per 100 cc.

It was also observed that when the other nonprotein nitrogen constituents of the blood decrease, the guanidine concentration falls more slowly than either the creatinine or urea. Consequently, in these cases a relatively normal blood urea and creatinine may be present with a high guanidine concentration. This is well shown in Case 12. In fact the data presented in Tables I and II point to the probability that an increase in blood guanidine, in renal insufficiency, is largely due to failure of excretion by the kidney.

In the series of cases in Table I, there seems to be no direct correlation between the degree of hyperguanidinemias and the blood pressure. Extremely high blood pressures may accompany low guanidine values. Case 48 showed a blood guanidine concentration of 2.28 mg with a blood pressure that is

fifteen minutes and take a capsule containing the vaccine. This vaccine consisted of 10 billion heat killed typhoid bacilli contained in a gelatin capsule mixed with starch.\* An hour later he was permitted to eat breakfast. The second morning he was instructed to take only the vaccine capsule one hour before eating. The same procedure was carried out on the third day as on the second, the bile capsule being given only on the first day. This same method of administration was used in every case in which the oral vaccine was given. As shown by the table, the agglutinin concentration in the blood gradually increased each week up to and including the fourth week following the completion of the vaccination.

The average agglutinin titer at the end of the fourth week was 1:604 as compared to an agglutinin titer of 1:496 in the case of the group vaccinated subcutaneously. As it takes only three days to administer the vaccine orally,

TABLE IV

SUBCUTANEOUS ADMINISTRATION OF TYPHOID VACCINE TO INDIVIDUALS GIVING A HISTORY OF PREVIOUS VACCINATION AGAINST TYPHOID

NUMBER OF PATIENTS IN EACH GROUP	PREVIOUS VACCINATION HISTORY	AGGLUTINATION TITER BEFORE VACCINATION	AGGLUTINATION TITER 4 WEEKS AFTER LAST INJECTION OF VACCINE
23	This group gave a history of a previous subcutaneous vaccination against typhoid varying from 1½ years to 10 years previous to this present vaccination with an average of 6.2 years.	Only one of this group gave a positive agglutination titer before vaccination, this one being 1-40.	1-320
30	This group gave a history of a previous vaccination against typhoid varying from 2½ years to 18 years. An average of 5-6 years.	Only 4 of this group gave an agglutination titer before vaccination. Two gave 1-40 and two 1-80.	1-640
Total 53 patients		Average agglutination before vaccination 1-7.	Average agglutination for all groups 4 weeks after vaccination 1-503

those vaccinated in that way attained the peak of protection approximately two weeks ahead of the group vaccinated subcutaneously.

For this experiment we secured 71 persons, all but one of whom gave a history of previous vaccination against typhoid, given subcutaneously. This one person had been given the vaccine orally the year before. These 71 people were given the vaccine orally as described above and their blood serum tested for agglutinins just before the vaccine was taken and four weeks after the completion of the course of oral vaccine.

The 53 persons in this group all gave a history of previous vaccination against typhoid as indicated. We administered the vaccine subcutaneously in the usual manner after testing their blood serum for agglutinins before administering the first dose. The agglutinin titer four weeks after the last injection of vaccine showed an average of 1:503 for this group as compared with an agglutinin titer of 1:710 for the orally vaccinated group.

\*Lilly's Typhoral, a commercial preparation, was the vaccine used.

those for either urea or creatinine, the similarity in variation suggests that the concentration of guanidine (like urea, uric acid and creatinine) depends largely upon the degree of renal insufficiency.

3 No direct relationship was found between the degree of hypertension (in kidney diseases) and the blood guanidine concentration.

4 Eleven out of 12 cases of hypertension without nitrogen retention showed definitely normal values for blood guanidine. The one case that did show a hyperguanidemia was later shown to be a case of arteriosclerotic Bright's disease. All this strongly points to hyperguanidemia being closely related to the renal pathology itself, rather than to the hypertension that may be present.

#### REFERENCES

- 1 Major, R. H. The Possible Relationship Between Guanidine and High Blood Pressure. *Am J M Sc* 170: 228, 1925.
- 2 Major, R. H. The Excretion of Guanidine Bases in Two Cases of Arterial Hypertension With Reduction of Blood Pressure, *Bull Johns Hopkins Hosp* 36: 757, 1925.
- 3 Major, R. H. The Effects of Guanidine Compounds on the Blood Pressure When Introduced Slowly Into the Circulation and Into the Gastrointestinal Tract. *Bull Johns Hopkins Hosp* 39: 215, 1926.
- 4 Major, R. H. Studies on the Effect of Hepatic Extract Upon Experimental Hypertension Produced by Guanidine Compounds, *Bull Johns Hopkins Hosp* 39: 222, 1926.
- 5 Greenwald, I. The Supposed Presence of Methyl Guanidine in the Blood of Parathyroidectomized Dogs, *Quart J Exper Physiol* 16: 347, 1926.
- 6 Greenwald, I. The Solubility of Some Pierates and the Determination of Guanidines in Urine, *Biochem J* 20: 665, 1926.
- 7 White, F. D. A Note on the Nature of the Pierate Obtained From Normal Urine by the Method of Findlay and Sharpe. *J Biol Chem* 71: 419, 1926.
- 8 Pfaffner, J. J., and Myers, V. C. Colorimetric Estimation of Methyl Guanidine in Biological Fluids, *Proc Soc Exper Biol & Med* 23: 830, 1926.
- 9 Major, R. H., and Weber, C. J. The Probable Presence of Increased Amounts of Guanidine in Blood of Patients With Arterial Hypertension, *Bull Johns Hopkins Hosp* 40: 85, 1927.
- 10 Weber, C. J. The Determination of Guanidine Bases in the Blood, *Proc Soc Exper Biol & Med* 24: 712, 1927.
- 11 Major, R. H., and Weber, C. J. Possible Increase of Guanidine in the Blood of Certain Persons With Hypertension, *Arch Int Med* 40: 891, 1927.
- 12 Nakazawa, F., and Abe, S. Über das Wesen der blutdrucksteigernden wirken des Guanidines, *Tohoku J Exper Med* 11: 308, 1928.
- 13 Major, R. H., and Weber, C. J. The Effect of Guanidine Compounds on Unanesthetized Dogs, *J Lab & Clin Med* 15: 125, 1929.
- 14 Major, R. H. Blood Chemistry Studies on Arterial Hypertension. *Am J M Sc* 177: 188, 1929.
- 15 Major, R. H. Chemical Factors Regulating Blood Pressure, *Am J M Sc* 183: 81, 1932.
- 16 Pfaffner, J. J., and Myers, V. C. On the Colorimetric Estimation of Guanidine Bases in the Blood. *J Biol Chem* 87: 345, 1930.
- 17 Remond, A., and Colombes, H. Investigaciones sobre el metabolismo de la guanidina. *Ars Med Barcelona* 6: 71, 1930.
- 18 de Wesselow, O. L. V. S., and Griffith, W. J. Blood Guanidine in Hypertension. *Brit J Exper Path* 13: 428, 1932.
- 19 Turries, J. Sur la guanidine du sang dans les affections hépatiques et l'insuffisance rénale. *Compt rend Soc de biol* 112: 658, 1933.
- 20 Pekelis, L. Contribution à l'étude de la concentration des bases guanidiniques dans le sang et dans le liquide céphalo rachidien au cours de divers états pathologiques, *Gaz d'hop* 106: 1417, 1933.
- 21 Pekelis, E., and Parenti, P. Ricerche sulle guanidinemia. I. Ulteriore contributo alla conoscenza del comportamento clinico della iperguanidinemia. *Riv di clin med* 35: 849, 1934.
- 22 Parenti, P., and Pekelis, E. Ricerche sulla guanidinemia. Il tasso della guanidina, della creatina e creatinina, dell'urea, dell'indacina, della reazione zantoproterica e dei fenoli del sangue in varie affezioni morbose, *Riv di clin med* 34: 673, 1933.
- 23 Parenti, P. Studi sulle guanidina sull'eliminazione urinaria dei basi guanidiche con speciale riguardo ai nefropazienti e agli ipertesi essenziali, *Riv di clin med* 37: 83, 1936.

As shown by Tables V and VI, we made a comparative study of the agglutinin titer of 26 persons before and four weeks after having been vaccinated against typhoid fever orally and 17 persons vaccinated subcutaneously. None of these 43 persons had ever been vaccinated in any manner against

TABLE VI  
SUBCUTANEOUS ADMINISTRATION OF TYPHOID VACCINE TO INDIVIDUALS GIVING  
NO HISTORY OF PREVIOUS VACCINATION

NO. OF PATIENTS IN EACH GROUP	AGGLUTINATION TITER BEFORE VACCINATION	AGGLUTINATION TITER FOUR WEEKS AFTER RECEIVING LAST INJECTION OF SUBCUTANEOUS VACCINE
3	One of these three gave an agglutinin titer of 1:40; the other two showed no agglutination before vaccination	1:640
14	0	1:320
Total in this group 17	Average agglutinin titer before vac- cination 1:2	Average agglutinin titer after vac- cination 1:312

typhoid previous to this time. Since most of our subjects were either medical students or nurses, it was difficult to get a very large number of persons who had had no previous vaccination.

The orally vaccinated group gave an agglutination titer of 1:320 and the subcutaneously vaccinated group a titer of 1:312, practically equal. A comparison of these titrations with the titrations obtained, using the serum of the groups giving a history of a previous vaccination, is interesting in that it shows that the groups giving a history of a previous vaccination gave a higher titer of agglutinins than did the ones with no previous vaccination. This result was of course to be expected. Agglutinin titrations in all cases were determined, using a twenty-four-hour broth culture of living typhoid organisms, Strain 4221 (American Type Culture Collection) Hopkins R. B. typhosus as an antigen.

The 107 people to whom we gave the oral vaccine lost no time from their usual activities. They reported no reaction, and we observed none except in three cases in which the act of swallowing the capsule produced some nausea. We did not blame the vaccine for this. The administration of the vaccine was under our direct supervision, hence, we had an opportunity of observing them all closely. The 80 people to whom we gave the subcutaneous vaccine all had the usual local reaction and about 50 per cent experienced a general systemic reaction. Three students received such a severe reaction that they lost several days of school.

During the past eight years we have been administering typhoid vaccine subcutaneously to all of the medical students as a routine prophylactic measure. Each year at least three students lose time from school because of a systemic reaction due to the vaccine. We have had no such experience with the oral vaccine.

#### SUMMARY

This study was carried out, using 187 human beings as subjects. The people used were divided into groups as shown by the tables. Agglutination titrations were made in all cases before the vaccine was administered and again four weeks after the last dose.

TABLE I

HYPODERMIC ADMINISTRATION OF TYPHOID VACCINE

Titration of agglutinin antibodies at weekly intervals Vaccine given subcutaneous, started Feb 16, 1930

PATIENT	HISTORY OF PREVIOUS VACCINATION	AGG TITER FEB 27 2ND DOSE	AGG TITR MARCH 2 2ND DOSE	AGG TITER MARCH 9	AGG TITER MARCH 16	AGG TITER MARCH 23	AGG TITER MARCH 30	AGG TITER APRIL 6	AGG TITER APRIL 13
1	None	No aggl	140	180	1160	1160	1320	1640	1320
2	2 yr ago	No aggl	180	1160	1160	1120	1640	1320	1320
3	10 yr ago	No aggl	180	1160	1160	1320	1320	1320	180
4	7 yr ago	No aggl	180	1160	1160	1640	1640	1320	1160
5	6 yr ago	140	180	1160	1320	1320	1160	1160	180
6	7 yr ago	140	180	1160	1320	1640	1320	1160	1160
7	4 yr ago	No aggl	1160	1320	1320	1640	1640	1320	1320
8	2½ yr ago	No aggl	140	180	1320	1320	1640	1640	1320
9	None	140	180	1160	1320	1640	1640	1320	1320
10	Typhoid fever 13 yr ago	No aggl	180	1160	1160	1320	1640	1320	1160
Average agglutination	18 yr ago	116	180	1160	1236	1400	1496	1352	1224

As shown by Tables V and VI, we made a comparative study of the agglutinin titer of 26 persons before and four weeks after having been vaccinated against typhoid fever orally and 17 persons vaccinated subcutaneously. None of these 43 persons had ever been vaccinated in any manner against

TABLE VI  
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During the past eight years we have been administering typhoid vaccine subcutaneously to all of the medical students as a routine prophylactic measure. Each year at least three students lose time from school because of a systemic reaction due to the vaccine. We have had no such experience with the oral vaccine.

#### SUMMARY

This study was carried out, using 187 human beings as subjects. The people used were divided into groups as shown by the tables. Agglutination titrations were made in all cases before the vaccine was administered and again four weeks after the last dose.

c.c. and 500 million per c.c. each of para typhoid A and B bacilli, suspended in saline, heat killed and preserved with merthiolate. The results obtained confirm those of other workers, namely, the maximum protection as indicated by the concentration of agglutinin antibodies in the blood was reached in the fourth week after the completion of the vaccination; therefore, from the beginning of the vaccination it took six weeks for the individual to reach a peak of protection.

As shown by Table II, we have 10 other persons to whom the typhoid vaccine was given orally. Agglutination titrations were made at weekly intervals as in the case of the 10 people of Table I. Oral vaccine was given as follows. The patient was instructed to report for his vaccine one hour and fifteen minutes before eating breakfast. A capsule of bile was given him to swallow with a small amount of water. He was then instructed to wait

TABLE III

ORAL ADMINISTRATION OF TYPHOID VACCINE TO INDIVIDUALS GIVING A PREVIOUS HISTORY OF VACCINATION AGAINST TYPHOID FEVER

NUMBER OF PATIENTS IN EACH GROUP	VACCINATION HISTORY	AGGLUTINATION TITER BEFORE VACCINATION	AGGLUTINATION TITER 4 WEEKS AFTER LAST DOSE OF VACCINE
2	One of these persons had just completed a course of typhoid vaccine given subcutaneously 4 months ago. He also gave a history of completing 5 previous courses administered subcutaneously. The other individual gave a history of one course of typhoid vaccine given subcutaneously one month previous to the present one.	One of these individuals' agglutination titer was 180 and the other 160.	1 3560
10	3 of these people gave a history of 2 or more courses of typhoid vaccine administered subcutaneously. The other 7 gave a history of one previous vaccination for typhoid administered subcutaneously from one month to 3 years previously. An average of $1\frac{1}{2}$ years.	8 of this group had an agglutination titer of from 140 to 1160. The agglutination titer of 2 was 0.	1 1280
35	All of these 35 persons had had one course of vaccine for typhoid administered subcutaneously except one who had taken oral vaccine 1 year previously and one who had had 2 previous courses given subcutaneously.	12 of this group gave an agglutination titer of from 140 to 1100. The other 23 persons showed no agglutinins in their blood.	1 640
24	The previous vaccination in this group varied from 4 months to 12 years ago. One who had been vaccinated 2 years previous to this vaccination also had had typhoid 9 years previous to that vaccination.	3 of this group gave an agglutination titer of 140, one of 1160, and the other 20 showed no agglutinins.	1 320
Total 71		Average 158	Average of all groups 1710

the anesthetic. The dose of pentothal was 40 mg. per kilogram. The sugar values were determined by the Somogyi micromethod for blood.<sup>6</sup>

TABLE II

THE TIME CURVE FOR THE BLOOD SUGAR LEVEL AFTER PENTOTHAL, 40 MG. PER KG. I. P.  
NORMALLY FED RABBITS

	CONTROL	20 MIN.	1 HR.	2 HR.	3 HR.
No. of observations	41	49	47	48	46
Mean	111	125	115	111	100
Mean deviation	19	22	23	20	13
Mean deviation of the mean	3.0	3.2	3.1	2.9	1.8

TABLE III

THE TIME CURVE FOR THE BLOOD SUGAR LEVEL AFTER PENTOTHAL, 40 MG. PER KG. I. P.  
RABBITS STARVED EIGHTEEN TO TWENTY-FOUR HOURS

	CONTROL	20 MIN.	1 HR.	2 HR.	3 HR.
No. of observations	47	48	47	47	47
Mean	87	96	81	86	82
Mean deviation	11	17	10	13	13
Mean deviation of the mean	1.7	2.1	1.5	2.0	2.0

It will be noted that the blood sugar level shows a definite rise at the twenty-minute interval. This period corresponds to the time of deepest depression for pentothal. With nembutal there was no mean change in the sugar level at the time of deepest depression, but there was an increased variability, some animals showing a rise, some a fall and others no change. A similar large variability has been shown after absinthe (Hrubetz and Pike<sup>7</sup>), another drug acting on the central nervous system. With pentothal, there is, after twenty minutes, a gradual return to the normal level with a drop at the three-hour interval. These changes are observed in both the normally fed and the starved groups. The values obtained at the three-hour interval represent the sugar levels at the time of recovery. The drop at this stage was also found after nembutal.

Since it is generally accepted that these short-acting barbiturates are detoxified in the liver, and since inanition lengthens the duration of their action, inanition may alter in some way the rate at which this detoxification takes place. Experiments for studying the correlation between liver function and barbiturate susceptibility are now in progress.

## SUMMARY

1. Pentothal sodium (sodium ethyl-1-methyl-butyl thiobarbituric acid) depresses the central nervous system producing stages varying from hypnosis to general anesthesia, depending on the dosage. The action is relatively short.

2. Fasting for twenty hours increased the susceptibility of rabbits to pentothal, and lengthened the duration of the anesthesia.

3. The influence of pentothal on carbohydrate mobilization was studied. No correlation was found between the blood sugar level and the susceptibility to the drug. Time curves were made depicting the changes in the blood sugar levels during the anesthesia and at the time of recovery after pentothal.

We do not claim from these results that the oral vaccine has any advantage over the subcutaneous as regards antibody response. It is highly probable that with a greater number in each group the agglutinin titers would be about the same or even reversed. The fact that the peak of the protection as indicated by the agglutinin titer, using the oral vaccine, is reached two weeks sooner than the subcutaneous is to be considered an advantage, provided the protection is as effective and lasting as that produced by the use of subcutaneous vaccination. We are of the opinion that since the antigens contained in the oral are the same as those in the subcutaneous vaccine the protection should be as effective. As regards the various antigens contained in the typhoid organisms there are the H, O and Vi antigens. Due to the fact that both the subcutaneous and oral vaccines used were heat killed we are dealing with only the O antigens. Felix and Pitt<sup>5</sup> state that the Vi antigen is heat labile. Rabbits immunized with avirulent strains or with heat killed virulent smooth strains produce O antibodies only. This suggests to us that

TABLE V

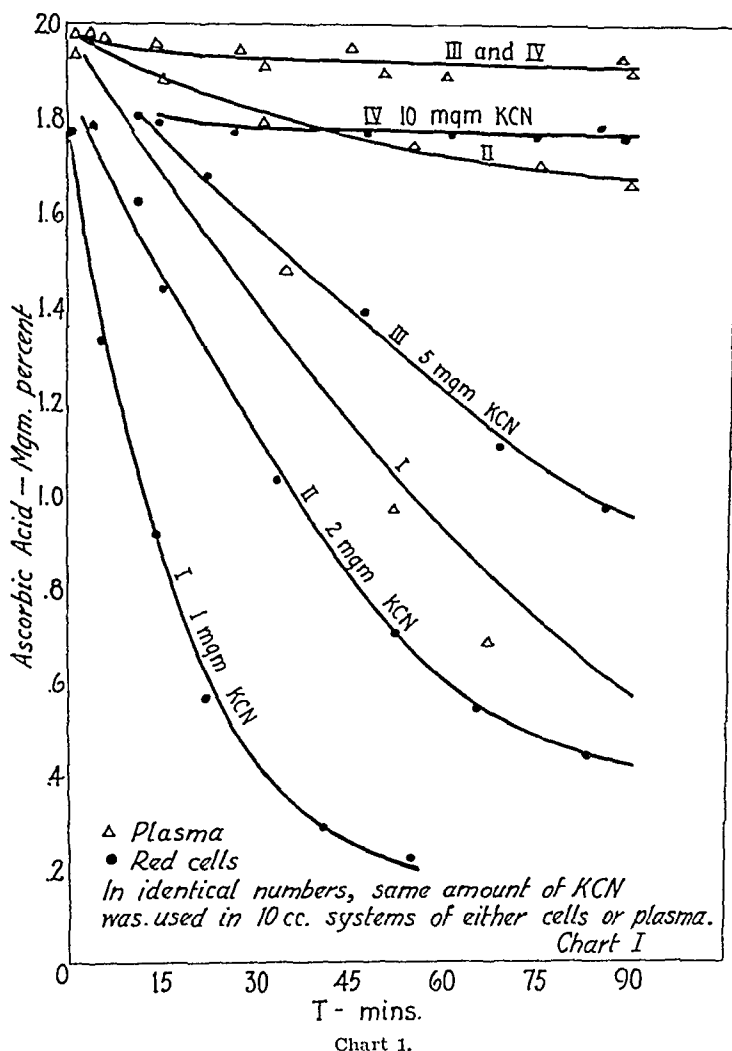
ORAL ADMINISTRATION OF TYPHOID VACCINE TO INDIVIDUALS WITH NO HISTORY OF PREVIOUS VACCINATION AGAINST TYPHOID

NO OF PATIENTS IN EACH GROUP	AGGLUTINATION TITER BEFORE VACCINATION	AGGLUTINATION TITER FOUR WEEKS AFTER RECEIVING LAST DOSE OF ORAL VACCINE
1	0	1 1280
2	0	1 640
13	0	1 320
10	Two of this group had an agglutination titer of 1 80, the rest showed no agglutinins	1 160
Total 26	Average titer for total before vaccination 1 6	Average titer for group after vaccination 1 320

some other manner of killing the typhoid bacilli for vaccines should be investigated. Also the serum of individuals vaccinated orally and subcutaneously should be tested by the mouse protection method and a comparison made.

We realize that the presence of agglutinin antibodies in the blood serum alone does not connote immunity, as the agglutinin reaction does not kill the bacteria or reduce their virulence. Furthermore, it is a well established fact that the agglutinin antibodies called forth in the blood by the vaccine or even having the disease disappear on the average in about six months following vaccination or having the disease. On the other hand, the immunity after vaccination with subcutaneous vaccine lasts on the average two to three years. Only time, therefore, and experiments of the nature suggested above can definitely prove whether the immunity gained after oral vaccination is as effective and lasting as that produced by giving the vaccine subcutaneously. The work of Claver,<sup>4</sup> Pirie and Orenstein,<sup>11</sup> and others in South Africa, and Gauthier<sup>8</sup> in Greece using large numbers of persons as subjects for oral vaccination, seems to point to the conclusion that the oral vaccine is an economic and effective prophylactic measure against typhoid as regards length of protection and reduction in incidence of the disease.

Blood immediately after collection is transferred to a test tube containing 10 mg. of KCN and 15 mg. of potassium oxalate. This is more than adequate for 10 c.c. of blood. In studying the red cell value as well as the plasma value, a hematocrit is taken or the volume noted so that the final volume can be made up to the original one. The plasma is separated by centrifugalization. To 2 c.c. of plasma is added 2 c.c. of distilled water and 6 c.c. of 10 per cent



metaphosphoric acid (made up fresh daily). The mixture is stirred for thirty seconds and allowed to stand for three minutes. In order to separate the protein precipitate from the protein free fluid, either filtration (using Whatman 42 f.p.) or centrifugalization is satisfactory. Two cubic centimeters of either the filtrate or the supernatant fluid is used for the determination of ascorbic acid and placed in a 50 c.c. beaker for titration with 2.6 dichlorophenol indophenol. Titrations to a pink end point are carried out using a light with

The first group of 20 individuals was divided so that 10 received the oral and 10 the subcutaneous vaccine. Agglutinin titers were run on the serums of these two groups at weekly intervals after the last dose of vaccine until the titer began to drop. The vaccination history and the history of having had the disease was secured from each case. Results are shown in the tables.

#### CONCLUSIONS (IN THIS SERIES OF CASES)

1 Typhoid vaccine administered orally produces as great or greater concentrations of agglutinin antibodies in the blood serum of human beings as the typhoid vaccine administered subcutaneously.

2 The oral vaccine brings about this concentration of agglutinin antibodies in a shorter length of time.

3 No observed or reported reaction followed the administration of the oral vaccine.

4 Severe reactions causing loss of time from work do occur when the vaccine is given subcutaneously.

5 People in general take the oral vaccine more willingly than they do the subcutaneous.

6 The oral vaccine is more easily administered because it requires less equipment and preliminary preparation.

7 Economically and practically the oral vaccine is more desirable than the subcutaneous.

#### REFERENCES

- 1 Arnold L. Alterations in Endogenous Enteric Bacterial Flora and Microbic Permeability of the Intestinal Wall. *J Hyg*, 29: 82, 1929.
- 2 Besredka A. Immunisation locale, *Presse méd* 32: 585, 1924.
- 3 Burke V. and Barnes I. Typhoid Vaccination by Mouth. *J Infect Dis* 39: 67, 1925.
- 4 Cluver F. Oral Immunization Against Typhoid in South Africa, *Lancet* 216: 1302, 1929.
- 5 Felix A. and Pitt R. Murphree. A New Antigen of *B. typhosus*. *Lancet* 2: 186, 1934.
- 6 Finner, J. G. and Simons J. Oral Vaccination. *Illinois M. J* 61: 21, 1932.
- 7 Garbat A. L. The Oral Method of Prophylactic Typhoid Immunization, *Med. J. & Rec* 126: 57 and 112, 1928.
- 8 Gauthier A. Vaccination by the Mouth Against Typhoid. *Bull Acad de med. Paris* 91: 472, 1924. *Abst. J. A. M. A.* 82: 1996, 1924.
- 9 Hoffstadt, Rachel E., Thompson, Randall L., and Martin, Carl L. Immunological Studies of Typhoid Vaccine by Mouth. *Am. J. Hyg* 9: 27, 1929.
- 10 Pijper Adriaans and Draai, Helen. Typhoid Agglutination After Oral Immunization. *Brit. J. Exper. Path.* 11: 112, 1930.
- 11 Price, J. H., and Orenstein A. D. Administration of Vaccine Per Os in an Outbreak of Enteric Fever. *Med. J. South Africa* 18: 224, 1923.
- 12 Simons J. Absorption of Antigens From Body Surfaces, *Proc. Soc. Exper. Biol. & Med.* 29: 20, 1931.
- 13 Tuft L., Yagle L. and Roger, S. Comparative Study of Antibody Response After Administration of Typhoid Vaccine With Particular Reference to Intradermal and Oral Methods, *J. Infect. Dis.* 50: 98, 1932.

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## REFERENCES

1. Van Eekelen, M., Emmerie, A., Josephy, B., and Wolff, K. L.: Über Vitamin C in Körperflüssigkeiten, *Klin. Wehnschr.* 13: 564, 1934.
2. Schneider, E., and Widman, E.: Blutserum, Bluteiweisskörper und Vitamin C, *Klin. Wehnschr.* 142: 1454, 1935.
3. Heinemann, M.: On the Partition of Ascorbic Acid Between Plasma and Erythrocytes, *Acta Brevia Neer. Phys.* 6: 159, 1936.
4. Tillmans, J., Hirsch, P., and Hirsch, W.: Das Reduktionsvermögen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C; der reduzierende Stoff des Citronensaftes, *Ztschr. f. Untersuch. d. Lebensmitt.* 63: 1, 1932.
5. Farmer, C. J., and Abt, A. F.: Ascorbic Acid Content of Blood, *Proc. Soc. Exper. Biol. & Med.* 32: 1625, 1935.
6. Fujita, A., and Iwatake, D.: Über die Bestimmung von Vitamin C mittels 2,6-Dichlorphenol-indophenol, *Biochem. Ztschr.* 277: 293, 1935.
7. Cohen, B., Gibbs, H. D., and Clark, W. M.: Studies on Oxidation-Reduction; Preliminary Study of Indophenols, *Public Rep.* 39: 804, 1924.
8. Pijoan, M., Townsend, S. R., and Wilson, A.: The Determination of Reduced Ascorbic Acid in Blood, *Proc. Soc. Exper. Biol. & Med.* 35: 224, 1936.
9. Barron, E. S. G., de Meio, R. H., and Klemperer, F. J.: Studies on Biological Oxidations; Copper and Hemochromogens as Catalysts for Oxidation of Ascorbic Acid. Mechanism of Oxidation, *J. Biol. Chem.* 112: 625, 1936.
10. Pijoan, M., and Klemperer, F.: Determination of Blood Ascorbic Acid, *J. Clin. Investigation* 16: 443, 1937.

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LOBAR PNEUMONIA AND ORGANIC HEART DISEASE\*

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CONFLICTING statements appear in the literature as to the incidence of lobar pneumonia in individuals with organic heart disease. The following statement is made in the chapter on lobar pneumonia in Osler and McRae, *Principles and Practice of Medicine*:<sup>1</sup> "The wards and postmortem room show a very striking contrast in their pneumonia statistics, owing to the occurrence of what may be called terminal pneumonia. During the winter months, patients with heart disease are not infrequently carried off by pneumonia which may give few or no signs. It is nearly always of the lobar form." Cecil and Plummer<sup>2</sup> claim that a terminal lobar pneumonia is seen very frequently in the course of chronic cardiac and renal diseases. In contrast, Austrian in his article on acute lobar pneumonia in Tice's *Practice of Medicine*<sup>3</sup> states: "In chronic diseases pneumonia is a very usual terminal event, but it is much more frequently of the lobular than the lobar variety." Cohn and Lewis<sup>4</sup> note that of 1,456 patients with lobar pneumonia admitted to the Hospital of the Rockefeller Institute only 3.7 per cent had coincident organic heart disease.

This study was made to determine the frequency with which lobar pneumonia is found at autopsy in adult patients who had organic heart disease. It was felt that this approach to the problem would give more accurate information than an attempt to determine the incidence of coincident heart disease in clinical lobar pneumonia, since patients are often too ill to have a complete

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pression of the respiration is dependent upon the amount of the drug in the circulating blood and is negligible when therapeutic amounts are administered. When the pentothal is given intravenously, even very small doses may produce depression or cessation of respiration, if the injection is made too rapidly. On the other hand, if the rate of injection is regulated, so as to give the desired degree of depression, pentothal may be administered in relatively huge doses over a prolonged period without producing respiratory embarrassment.

#### EFFECTS OF INANITION

If rabbits were given water *ad lib* but deprived of food for twenty hours, they were much more susceptible to the depressant action of pentothal. The induction of the anesthesia from 40 mg per kilogram given intraperitoneally, was rapid and smooth, only about five minutes being required to develop anesthesia. The duration of the action was definitely longer in the starved animals than in those which were normally fed. In the starved group, consciousness was regained only after an hour (average time) and complete recovery did not occur until two and one half to three hours after the injection. Several animals remained fully anesthetized for more than the three hour observation period. With the dosage indicated, only 31 per cent of the normally fed rabbits became fully anesthetized with good relaxation, whereas 62 per cent of the starved group showed the same reaction.

TABLE I  
DURATION OF DEPRESSION AFTER PENTOTHAL (40 MG PER KILO)

	NORMALLY FED	STARVED
No. of observations	98	96
Per cent of animals anesthetized	31	62
Mean time of onset	5 min	5 min
Mean time to beginning of return of consciousness	40 min	60 min
Mean time to complete recovery	85 min	135 min

Although pentothal has only recently been made available for practical use, numerous clinical reports have appeared in the literature. The work of all corroborate our experimental findings, Lundy,<sup>3</sup> Jarman and Abel,<sup>4</sup> Horsley.<sup>5</sup> They all emphasize the smooth induction, fair relaxation, short duration, and absence of postoperative restlessness. They also report some depression of the respiratory center producing a change in the depth rather than any change in the rate of breathing.

#### CARBOHYDRATE MOBILIZATION

In order to determine whether the constancy of the blood sugar level during the height of the depression and the fall at the time of recovery are specific for nembutal, or are characteristic for other short acting barbiturates, it was thought advisable to follow the blood sugar time curve throughout the period of anesthesia and recovery from pentothal. Samples of blood were obtained from the marginal ear vein of the rabbits before the intraperitoneal injection and at twenty minutes, one, two, and three hours after the administration of

contain higher cellular values when anticoagulant salts were employed. In oxalated blood, the average of 12 determinations showed 34.5 mg. per cent urea in plasma and 26.4 mg. per cent urea in the cells, plasma containing on the average about 31 per cent more urea than the red cells; and the average cellular urea was about 77 per cent of that in plasma. This approximates the commonly accepted view of the distribution of urea between cells and plasma. It is important to recognize that such a partition occurs only in blood containing anticoagulant salts and that even in such blood there is a wide variation from the average, as seen in Table I.

In routine laboratory work, samples of blood frequently stand for several hours, usually in the ice box, before urea determinations are made. Hence the *effect of standing* at ice box temperatures on the partition of urea was determined. Fourteen samples of blood containing various anticoagulants were analyzed at various intervals up to forty-eight hours after removal from the patient. There was found an ebb and flow of the urea between cells and plasma in defibrinated, heparinized and hirudinized blood but no consistent changes on standing for two to three days in the ice box. In oxalated blood, the urea content of plasma tended to increase and of the red cells to decrease after twenty-four to forty-eight hours. Four examples of the changes in oxalated blood have been depicted in Table II.

TABLE II

THE EFFECT OF STANDING ON THE PARTITION OF UREA IN OXALATED BLOOD

<i>Example 1</i>					<i>Example 3</i>				
Time (hours)	0	6	24	48	Time	0	4	8	24
Volume of red cells per cent	40.0	40.2	43.7	46.3	Volume of R.B.C.	39.0	39.1	39.5	41.1
Plasma urea (mg. per cent)	42.0	37.5	38.4	34.1	Plasma urea	42.7	41.2	27.2	31.9
Red cell urea (mg. per cent)	20.6	21.5	42.0	26.0	Red cell urea	21.8	22.5	45.9	32.4
<i>Example 2</i>					<i>Example 4</i>				
Time	0	4	8	24	Time	0	4	8	24
Volume of R.B.C.	42.1	42.4	43.3	47.1	Volume of R.B.C.	48.3	48.7	42.3	47.8
Plasma urea	28.2	28.2	28.4	21.5	Plasma urea	29.1	28.2	27.6	24.7
Red cell urea	21.6	21.8	23.0	25.0	Red cell urea	16.2	17.0	18.3	19.9

The results in Table II compared with those in Table I suggested that potassium oxalate and other anticoagulant salts may increase the concentration of urea in plasma at the expense of urea in the cells. On standing, a tendency to return to the original distribution apparently occurs. The *effect of anticoagulant salts* was determined in 20 samples of blood, half of each sample being heparinized or defibrinated and the other half treated with an anticoagulant salt. The results of this experiment are shown in Table III. Potassium oxalate and other anticoagulant salts invariably lowered the urea content of the red cells, compared with the values in defibrinated or heparinized blood. In practically all samples the urea content of plasma was simultaneously increased, the few instances in which the reverse occurred being probably attributable to experimental error.

REFERENCES

- 1 Blackberg S N and Hrubetz M C Some Effects of Pentobarbital on the Rabbit, *Proc Soc Exper Biol & Med* 34 (5) 1931
- 2 Hrubetz M C and Blackberg S N Factors Influencing Nembutal Anesthesia *Proc Soc Exper Biol & Med* 35 (6) 1931
- 3 Lundy J S Intravenous Anesthesia The Use of Two New Thiobarbiturates *Mayo Clinic Proc Staff Meetings* 10 (30) 1935
- 4 Jarman R and Allet A I Intravenous Anesthesia With Pentothal Sodium *Lancet* 1 422 1936
- 5 Horsley J S Pentothal Sodium in Mental Hospital Practice *Brit M J* 1 938, 1931
- 6 Somogyi M Quantitative Clinical Chemistry by Peters J P, and Van Slyke D D 2 p 468
- 7 Hrubetz M C and Pike F H Absinthe and the Blood Sugar Level *Proc Am Physiol Soc* March 1936 p 81

ASCORBIC ACID CONTENT OF RED CELLS AND PLASMA\*

M PIJOAN AND E EDDY BOSTON MASS

SINCE vitamin C has been definitely identified with ascorbic acid, the clinical importance of this substance has increased. Growing interest has developed in the study of the proportion of ascorbic acid between red cells and plasma in various diseases. In normal individuals van Eekelen, Emmerie Josephy and Wolff<sup>1</sup> found that ascorbic acid was evenly distributed between cells and plasma. On the other hand Schneider and Widman<sup>2</sup> and Heinemann<sup>3</sup> noted a higher ascorbic content in the red cell than in the plasma. Heinemann<sup>3</sup> further noted in a study of various diseases that the ascorbic acid in the red cells varied considerably reaching higher red cell values in proportion to the serum in patients with anemias or with peptic ulcers. However, the interpretation of blood ascorbic acid values is uncertain in part because of the variations in the values encountered in routine analyses. In attempting to assay ascorbic acid in both cells and plasma the methods of analysis must be considered. Tillmans, Hirsch and Hirsch<sup>4</sup> first developed the method of determining the presence of ascorbic acid (reduced) in serum. To make it clinically practical this original method was modified by Farmer and Abt<sup>5</sup>. They used as a deproteinizing agent metaphosphoric acid which had been introduced for this purpose by Fujita and Iwatike<sup>6</sup> and they determined the reduced ascorbic acid in the filtrate with 2,6-dichlorophenol indophenol.<sup>7</sup> Pijoan, Townsend and Wilson<sup>8</sup> noted a considerable loss of ascorbic acid in the serum by this method. This rapid loss of ascorbic acid is due as Barron, de Meio and Klemperer<sup>9</sup> have pointed out to copper and hemochromogens. A loss, but to a lesser degree is found also in the method introduced by van Eekelen, Emmerie Josephy and Wolff,<sup>1</sup> as some of the ascorbic acid is lost in mercuric sulphide precipitation. Pijoan and Klemperer<sup>10</sup> have suggested the use of potassium cyanide to inhibit the disturbing catalysts responsible for oxidizing ascorbic acid. For purposes of simplicity and convenience the method is presented

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tion of all of the urea values according to cell volume failed to demonstrate any significant relation. The coincidental decrease in both volume and urea content of the red cells following addition of oxalate was the only instance in which one might conclude that the change in the urea content of the cells was due to shrinkage of the cells. Results in other experiments suggested that such was a fortuitous occurrence not indicative of a causal relationship. There must be factors other than the water content of the red blood cells which affect their urea content.

#### SUMMARY

Potassium oxalate and other anticoagulant salts decrease the urea content of the red blood cells and increase the urea content of plasma, the values tending to return toward the initial on standing. In defibrinated, heparinized and hirudinized blood the urea content of the red cells tends to be lower than that of plasma when the value in the latter is high, and vice versa. With any anticoagulant, the urea content of plasma is seldom the same as that of the red cells.

#### REFERENCES

1. Aszódi, Z.: Ueber den Harnstoffgehalt der roten Blutkörperchen, *Biochem. Ztschr.* 146: 343, 1924.
2. Boyd, E. M.: The Extraction of Blood Lipids, *J. Biol. Chem.* 114: 223, 1936; Boyd, E. M., and Murray, R. B.: The Effect of Anticoagulants on Blood Lipids, *J. Biol. Chem.* 117: 629, 1937.
3. Cohen, J. B.: Ueber die Verteilung des Harnstoffs in menschlichen Blut und in menschlichen Sekreten, *Biochem. Ztschr.* 139: 516, 1923.
4. Gad-Andresen, K. L.: Die Verteilung des Harnstoffs im Organismus, *Biochem. Ztschr.* 116: 266, 1921.
5. Heller, V. G., and Paul, H.: Changes in Cell Volume Produced by Varying Concentrations of Different Anticoagulants, *J. LAB. & CLIN. MED.* 19: 777, 1934.
6. Polonovski, M., and Auguste, C.: Réparation de l'urée dans le sang, *Compt. rend. Soc. de biol.* 87: 681, 1922; Étude sur la répartition de l'urée dans le sang (plasma et globules) et le liquide céphalorachidien, *J. de physiol. et de path. gén.* 21: 267, 1923.
7. Straube, G., and Hofmann, R.: Die normale Harnstoffkonzentration im Blut und Liquor cerebrospinalis, *Klin. Wchnschr.* 13: 1377, 1934.
8. Wu, H.: Separate Analysis of the Corpuscles and Plasma, *J. Biol. Chem.* 51: 21, 1922.

a daylight filter. A 1/1000 Molar solution (29 mg of dye in 100 cc water) is the most efficient for this purpose. The dye must be dissolved in water heated to 85° C, shaken for fifteen minutes and filtered if necessary. The details of standardization and calculation have been already presented by Pijoan and Klemperer<sup>10</sup>. Simple calculation is accomplished by multiplying the amount of dye used by 44 if 2 cc of plasma are assayed. The ascorbic acid value will then be in milligrams per cent. In assaying the ascorbic acid value of red cells, the red cells are washed four times with 0.9 per cent NaCl solution containing 100 mg per cent of KCN and separated each time by centrifugalization. After the four washings their volume is doubled by adding distilled water containing 100 mg per cent of KCN. The hemolyzed cells are then assayed in the same manner as the plasma. Whole blood can also be used and the red cell value computed by hematocrit proportion.

It is of interest to note that much more KCN is required to inhibit the action of the catalysts in the red cells than in the serum. In the following chart (Chart 1) the curves designated by the same number represent samples of red cells and plasma containing the same amount of KCN.

In a series of carefully controlled analyses of blood from normal individuals we were unable to find red cell values exceeding or equal to the plasma values.

TABLE I  
BLOOD ASCORBIC ACID VALUES IN NORMAL INDIVIDUALS

	ASCORBIC ACID CONTENT	
	RED CELLS	MG PER CENT PLASMA
E E	15	21
E R	10	164
R K	142	170
O B	17	20
W B	0.98	131
M T	12	151
D L	131	172
D A	152	198
P S	164	190
C B	112	156
K R	0.84	143
E E	120	280

Normal ascorbic values in the plasma of over 100 normal individuals were reported by Pijoan and Klemperer<sup>9</sup>.

Even when certain individuals raised their blood ascorbic acid value by consuming 0.25 gm of ascorbic acid, the plasma levels were always higher than the red cell values. We obtained plasma values as high as 3.2 mg per cent by this procedure.

From the foregoing data in Table I it is suggested that in normal individuals considerable variation exists between red cell values and plasma values. In the light of these findings considerable care must be taken before interpreting the blood ascorbic acid content in disease.

#### CONCLUSION

A method is introduced to study the ascorbic acid content of red cells. The ascorbic acid content appears to be higher in the plasma than that present in the red cells.

Total calcium determinations were made on trichloroacetic acid centrifugates by the Clark and Collip modification<sup>5</sup> of the Kramer-Tisdall method. An attempt was also made to determine nonprotein bound calcium by the method of von Berenesy<sup>6</sup> and Hermann;<sup>7</sup> however, this method was found to be unsatisfactory<sup>8</sup> and the results which had been obtained were discarded. Total inorganic phosphorus determinations were made on trichloroacetic acid centrifugates by the method of Fiske and Subbarow.<sup>9</sup> Surface tension and viscosity measurements were made on fresh serum at 27° to 28° C. by means of the Du Nouy tensiometer<sup>10</sup> and the Oswald viscosity tube, respectively. While it is realized that exact measurement of these physical properties by either of these methods is not obtained, the measurements obtained on different samples of serum from the same animal have a comparative value with each other, since they were made at the same temperature upon freshly separated serums, and in each case with the same piece of apparatus.

## RESULTS

The results of studies conducted on 12 cats and 6 dogs are briefly summarized in Table I. Since the variations which occurred in individual animals were small, the results are presented in the form of average values. In both control and digitalis-treated animals, the final sample of blood was taken at a time during which it had previously been shown that diuresis was in progress following the intravenous injection of 50 mg. per kg. of digitalis.

TABLE I

AVERAGES OF TOTAL CALCIUM, INORGANIC PHOSPHORUS, VISCOSITY AND SURFACE TENSION OF BLOOD SERUM FOLLOWING THE INTRAVENOUS ADMINISTRATION OF DIGITALIS (50 MG. PER KG.)

ANIMALS USED	AMOUNT OF BLOOD IN INITIAL SAMPLE (C.C. PER KG.)	TIME FROM INITIAL TO FINAL SAMPLE (MINUTES)	TIME FROM DIGITALIS INJECTION TO FINAL SAMPLE (MINUTES)	NORMAL (INITIAL SAMPLE)	AFTER BLOOD REMOVAL (FINAL SAMPLE)	AFTER BLOOD REMOVAL AND DIGITALIS (FINAL SAMPLE)
<i>Total Calcium</i> (mg. per 100 c.c. of serum)						
4 Cats	7.1	40		11.5	11.4	
5 Cats	7.2	67	44	11.6		11.2
2 Dogs	1.8	56		12.3	12.5	
4 Dogs	1.6	55	43	12.9		12.7
<i>Inorganic Phosphorus</i> (mg. per 100 c.c. of serum)						
3 Cats	9.0	55		4.9	4.7	
6 Cats	8.5	65	46	3.3		3.2
2 Dogs	1.8	56		3.9	3.9	
3 Dogs	1.3	58	45	3.6		3.5
<i>Viscosity</i> (Oswald tube—in seconds)						
2 Cats	9.2	55		128	131	
3 Cats	7.4	56	43	139		138
1 Dog	2.0	60		115	120	
2 Dogs	1.3	55	41	133		133
<i>Surface Tension</i> (Du Nouy Tensiometer)						
2 Cats	9.2	55		62.0	61	
4 Cats	8.0	72	47	60.7		59.0
1 Dog	1.6	53		61.0	60	
2 Dogs	1.3	55	41	58.5		60.5

cardiovascular survey. The material comprises all individuals with heart disease, aged twenty years and over, autopsied at the Louisville City Hospital from 1931 to 1936, a total of 465 cases. Table I comprises the etiologic types of heart disease encountered, distributed as to age and sex. Of the cases, 356 (76.5 per cent) were hypertensive and arteriosclerotic, 54 (11.6 per cent) were syphilitic, 32 (6.9 per cent) were rheumatic, and 23 (5.0 per cent) were of miscellaneous including unknown etiology. There were 298 (64.0 per cent) males and 167 (36.0 per cent) females. Eighty-four per cent of the patients were over forty years of age. The cases were approximately equally divided between the white and negro races.

TABLE I

465 CASES OF HEART DISEASE ANALYZED ACCORDING TO ETIOLOGY, AGE AND SEX

ETIO TYPE	20-29		30-39		40-49		50-59		60-69		70-79		80		TOTAL	PER CENT
	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
Hyper Arter	2		15	14	31	22	46	44	72	21	40	22	18	1	356	76.5
Syphilitic	4	2	7	4	17	1	12	1	5	0	0	0	1	0	54	11.6
Rheumatic		5			4	4		4	1	2	0	0	0	0	32	6.9
Miscellaneous	1	2	1	2	4	1	4	3	2	0	2	1	0	0	23	5.0
Total	10	12	26	2	56	28	66	52	80	23	42	23	19	6	465	

Of these 465 cases only 6 (1.2 per cent) had lobar pneumonia. In contrast, 157 patients (33.7 per cent) died with bronchopneumonia. Of the 6 patients with lobar pneumonia, 5 had hypertensive and 1 syphilitic heart disease. All were admitted for lobar pneumonia, and only one gave an antecedent history of congestive heart failure.

Since the age and sex incidence of lobar pneumonia might explain the infrequency with which it and heart disease were coincidentally found in this study, 743 consecutive patients with lobar pneumonia, aged twenty years and over admitted to the hospital, were analyzed as to these factors. It was found that 365 (49 per cent) were over forty years of age as compared with 84 per cent of the heart patients who were in this age group. Lobar pneumonia occurred predominately in the male (73 per cent) just as was found in the patients with heart disease. Thus the age and sex incidence of lobar pneumonia cannot explain the rare association of the two diseases. Neither was the racial incidence of any significance.

Theoretically lobar pneumonia should occur frequently in individuals with heart disease. Even though the exact pathogenesis of lobar pneumonia is not fully understood, an attack is usually attributed to lowered general resistance, and there is experimental basis for the view that local conditions in the lung, such as the catarrhal process or previously damaged tissue, favor development of pneumococci.<sup>1,2</sup> If this conception is correct, the lungs of individuals with heart disease should furnish a fertile soil for the growth of pathogenic organisms. This study does not lend support to these theoretical considerations.

## SUMMARY AND CONCLUSIONS

Only 1.2 per cent of 465 autopsied cases of organic heart disease aged twenty years or over died with lobar pneumonia. There was only one patient with an antecedent history of congestive failure. In contrast 33.7 per cent

## THE AMOUNT OF COMPLEMENT IN THE BLOOD IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS\*

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THE similarity between the clinical pictures of rheumatic fever and rheumatoid arthritis is often so marked as to cause confusion in differential diagnosis. The onset of rheumatoid arthritis may closely resemble that of rheumatic fever, commencing acutely, with fever, leucocytosis, and hot reddened joints. In both diseases there may be a history of previous attacks of tonsillitis or pharyngitis. The most important clinical signs differentiating rheumatic fever and rheumatoid arthritis are those referable to cardiac involvement which so often is a complication of the former condition. But in adults with no history of previous attacks, the diagnosis may be particularly difficult. The absence of cardiac involvement is not helpful, for at this age the arthritis is the most important clinical manifestation and the heart often escapes.

Since the cause of either of these diseases is still uncertain, the suspected etiologic agents being streptococci of various types, and in rheumatic fever also a virus,<sup>1</sup> there is no definite means of differentiation. For this purpose use has been made of various laboratory methods, the most valuable of which is the electrocardiogram. This, according to Cohn and Swift,<sup>2</sup> Rothschild, Sacks and Libman,<sup>3</sup> and Master and Jaffe,<sup>4</sup> discloses abnormalities in almost 100 per cent of all patients suffering from rheumatic fever, while in patients suffering from rheumatoid arthritis, according to Master and Jaffe, electrocardiographic changes are either absent or insignificant.

Serologic tests for the differentiation of rheumatic fever and rheumatoid arthritis have been employed by Cecil,<sup>5</sup> and Nicholls and Stainsby.<sup>6</sup> These authors make use of the agglutination reaction of a strain of streptococcus cultivated from blood of cases of rheumatic fever with the serum of these patients. More recently Stuart-Harris<sup>7</sup> studied the hemolytic-streptococcus-fibrinolysis in chronic arthritis and rheumatic fever, and found that the blood of patients with rheumatoid arthritis is susceptible to fibrinolysis, whereas the blood from patients with rheumatic fever is resistant.

We have used the amount of complement in the blood as a means of differential diagnosis. The present report comprises a study of 45 patients suffering from rheumatic fever and rheumatoid arthritis.

The method used for quantitative estimation of the complement is as follows:

The patient's serum is diluted with saline in the proportion 1:10. A series of test tubes is filled with increasing amounts of this diluted serum, beginning with 0.4 and up to 1.5 c.c. Saline solution is added to the tubes up to the equal

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TABLE III

THE EFFECT OF ANTICOAGULANT SALTS ON THE PARTITION OF UREA IN BLOOD COMPARED WITH DEFIBRINATION AND HEPARIN

ANTICOAGULANT SALT	UREA			
	PLASMA		RED BLOOD CELLS	
	NO SALT	SALT	NO SALT	SALT
<i>Defibrination</i>				
Pot Oxal	47.6	54.9	51.0	30.4
Pot Oxal	22.8	28.2	29.0	21.8
Pot Oxal	17.6	26.1	54.9	45.2
Ammon Oxal	17.6	39.1	54.9	30.3
Sod Oxal	19.5	27.4	49.5	42.0
Sod Citrate	16.5	26.0	50.2	42.5
<i>Heparin</i>				
Pot Oxal	22.1	28.2	21.0	17.0
Pot Oxal	22.8	28.2	29.0	21.8
Pot Oxal	23.9	22.6	25.7	20.5
Pot Oxal	24.0	24.4	22.5	21.2
Pot Oxal	25.7	26.5	43.9	42.0
Pot Oxal	32.7	41.2	28.0	22.5
Pot Oxal	30.1	47.6	35.0	29.5
Pot Oxal	42.0	27.5	35.6	21.5
Pot Oxal	40.4	42.9	42.0	26.5
Pot Oxal	51.3	52.3	41.6	28.4
Ammon Oxal	22.1	28.2	21.0	17.0
Ammon Oxal	24.7	39.1	43.9	30.3
Sod Oxal	23.3	27.4	44.1	42.0
Sod Citrate	24.9	26.0	43.3	42.5

It has thus been shown that potassium oxalate lowers the urea content of the red cells and increases that of plasma, so that samples of oxalated blood invariably contain more urea in plasma than in the red cells. The question arose as to whether or not the concentration of oxalate would further affect urea values. Blood for urea estimations usually contains from 0.2 to 0.3 per cent of potassium oxalate, but occasionally the concentration is greater for various reasons such as insufficient blood being obtained from the patient to sufficiently dilute the anticoagulant provided in the vessel used for collecting the blood. Hence urea was determined in cells and plasma of samples of the same blood containing from 0.2 to 1.5 per cent of potassium oxalate. The addition of 0.2 per cent potassium oxalate effected the changes described above but adding more of the salt had no further influence on the partition of urea, although a progressive shrinkage in the volume of the red cells took place as more of the salt was added. The addition of many times the required amount of heparin or of hirudin did not materially change the partition of urea.

In all of the previous experiments the volume of the red cells was simultaneously determined with the urea values. A casual examination of percentage volumes suggested that a direct relation existed between volume and urea content of the red cells. This was apparent chiefly in the results from experiments in which the urea values were compared in oxalated and heparinized or defibrinated blood. When, however, the results of time and concentration experiments were plotted, the urea values against the volumes of the cells, no relation whatsoever could be established between the two. Also the tabula

blood cells. In several cases, the complement titer was determined repeatedly at various intervals during the course of the attacks, and in some we observed an increase of the titer with improvement in the clinical condition. For example, a man (Table II, A, Case 3), aged thirty years, suffering from a second attack of rheumatic fever preceded by tonsillitis, showed at first a low complement titer (less than 0.15). After removal of the tonsils the clinical signs of activity disappeared and an increase of the complement to normal titer was found and remained so on successive examinations. A progressive increase to the normal level was found in Case 4 in Table II, A. In other instances, how-

TABLE II  
THE COMPLEMENT TITER IN RHEUMATIC FEVER

A. RHEUMATIC FEVER: ACTIVE STAGE				B. RHEUMATIC FEVER: INACTIVE STAGE			
CASE	AGE	DATE	COMPLEMENT TITER	CASE	AGE	DATE	COMPLEMENT TITER
1	18		0.14	1	16		0.05
2	26	5/30	0.13	2	--		0.06
		6/20	0.15*	3	21		0.05
		8/22	0.15	4	33		0.06
3	30	7/25	0.15	5	29	10/17	0.06
		8/22	0.06			10/31	0.06
		9/12	0.04	6	29		0.06
		10/24	0.05	7	16		0.05
		12/19	0.06	8	17		0.05
4	24	8/ 1	0.15	9	20		0.04
		8/15	0.15	10	28		0.05
		10/24	0.11	11	35		0.06
		11/ 7	0.13				
		11/21	0.08				
5	18	8/15	0.09				
6	18	9/19	0.1				
		9/24	0.07				
7	32	10/ 3	0.13				
		1/30	0.12				
8	18	10/24	0.08				
9	28	11/ 7	0.16				
		11/14	0.15				
		11/28	0.15				
		12/12	0.15				
		1/30	0.13				
10	22	1/ 9	0.25				
		2/13	0.15				
11	31	1/30	0.14				
12	20	2/ 6	0.14				
13	25	2/ 6	0.14				
		2/20	0.15				
14	30	2/13	0.14				

\*In most examinations determinations were not made beyond the figure of 0.15.

ever, the complement titer remained low even after the clinical manifestations of the disease had disappeared. In these patients there was a tendency to diminution of the sedimentation rate of the red blood cells, while the complement titer was still low. The following case may serve as an example. A female patient (Table II, A, Case 9), who had an old rheumatic valvular lesion, was suffering from many months from active carditis, mild but persistent polyarthrits and subfebrile temperature. The sedimentation rate was very rapid (15' by Linzenmaier's method) and the complement titer was found to be less than 0.15 in repeated determinations. After removal of the tonsils and drain-

## DIGITALIS DIURESIS AND CERTAIN BLOOD SERUM CHARACTERISTICS\*

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### DIGITALIS DIURESIS AND BLOOD CALCIUM

IN AN earlier paper,<sup>1</sup> it was reported that intravenous injections of digitalis, in comparatively large doses, consistently produced a diuretic response in anesthetized cats, an effect which was later confirmed on dogs.

Our knowledge of the relation of urine secretion to the action of digitalis on the heart, blood pressure, and kidney vessels is still imperfect. However, since various investigators<sup>2,4</sup> have reported a synergistic stimulant action of digitalis and calcium on the heart, and since the permeability of blood vessels and tissues is closely associated with calcium, it was decided to ascertain whether quantitative changes in blood calcium accompany the diuretic response to digitalis. Blood phosphorus determinations and serum viscosity measurements were also recorded, because of their relation to calcium metabolism and kidney filtration, respectively. Surface tension measurements were also made on the blood serum.

### EXPERIMENTAL

The experimental animals used were cats and dogs. The cats were on a milk diet supplemented with raw liver, the dogs were fed a balanced food preparation occasionally supplemented with raw meat and bones. All animals were fasted for eighteen hours preceding the experiment. The cats were anesthetized with sodium barbital, 300 mg per kg by stomach, and the dogs with pentobarbital sodium (sodium ethyl 1-methyl-butyl barbiturate), 30 mg per kg intraperitoneally. When anesthesia was complete blood was removed by heart puncture for the normal determinations of calcium, phosphorus, viscosity, and surface tension. The control animals then received an intravenous injection of 0.85 per cent saline, 1 cc per kg, the other animals were injected in the same manner with 1 cc per kg of a modified alcohol free 5 per cent tincture of digitalis, a dose of 50 mg of digitalis per kg, an amount which had been shown to invariably produce diuresis in anesthetized cats,<sup>1</sup> and which was found to produce a similar effect in anesthetized dogs. The digitalis preparation was injected into the saphenous vein at the rate of approximately 1 cc per minute. Since it had been found that diuresis occurred in ten to fifteen minutes and continued for about an hour following the injection of this amount of digitalis, the second sample of blood was removed by heart puncture about one hour after the control sample, or about three quarters of an hour after the digitalis injection.

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clinical conditions and have found a decrease to almost disappearance of the complement in "acute polyarthritis" and "rheumatic endocarditis." Schnabel found a decrease of the complement in 45 per cent of the patients suffering from acute joint diseases. As these authors have not differentiated between rheumatic fever and rheumatoid arthritis, their conclusions provide no aid in the diagnosis. The normal amount of complement found in the group of rheumatoid arthritis justifies the assumption that the complement-determination may serve as an aid in the differentiation between rheumatic fever and rheumatoid arthritis. The significance of this fact is evident from our introductory remarks in which we pointed out the possibility of differentiating, on the basis of this method, the active from the inactive state of rheumatic fever when the clinical signs are not definite. This is often the case when fever is absent and the cardiac manifestations are indefinite.

The diminution of complement in rheumatic fever and its significance in the etiology of the disease may be explained as follows. Schuetze and Scheller<sup>10</sup> more than thirty years ago found a decrease of complement in animals which were sensitized to red blood cells of another species. Bieling<sup>11</sup> found the same

TABLE IV  
MISCELLANEOUS GROUP WITH UNCERTAIN DIAGNOSIS

NO.	AGE	DATE	COMPLEMENT TITER	DIAGNOSIS
1	30	5/ 9	0.06	Acute polyarthritis
		5/16	0.08	
2	46	7/25	0.11.	Recurring polyarthritis in an
		8/29	0.1	acute episode
		9/26	0.13	
		10/17	0.1	
3	29	8/22	0.04	Recurring polyarthritis in an
		8/29	0.06	acute episode. Erythema
				nodosum
4	30	9/12	0.04	Recurring polyarthritis in an
		10/17	0.06	acute episode
		10/31	0.04	
5	26	10/24	0.11	Recurring polyarthritis
		11/ 7	0.05	
6	38		0.08	Recurring polyarthritis in sub-
				acute state
7	17		0.15	Recurring polyarthritis.
				Urticaria

phenomenon in animals sensitized to streptococci and tubercle bacilli. Recently P. and M. Pely<sup>12</sup> observed a decrease of complement in patients suffering from various allergic diseases, such as asthma, urticaria, etc. These findings suggested the present investigation to us. Clinical, experimental and pathologic evidence has been accumulated in recent years by the investigators of rheumatic fever, indicating that this disease may be an allergic manifestation of a focal infection.<sup>13-16</sup> Long-standing or repeated low-grade focal infection, in which streptococci of various types play a leading rôle, sensitizes various mesenchymal tissues, such as joints, tendons, heart and blood vessels. An acute infection, most commonly tonsillitis, acting on these sensitized tissues, brings about the acute attack of rheumatic fever, which is accompanied by a decrease of the complement titer in the blood. The antigen-antibody reaction uses up the com-

Examination of the table reveals that the total calcium level of the blood serum was about 1 mg per cent higher in dogs than in cats, and remained unchanged in both the control and digitalis treated animals of each group. The inorganic phosphorus of the serum also remained the same in each series. The viscosity and surface tension of the serum showed very little change from normal in either control or digitalis animals, and such variations as occurred were not constant in the same direction.

#### CONCLUSIONS

Intravenous administration of digitalis in diuretic amounts (50 mg per kg) to anesthetized cats and dogs produced no significant quantitative changes in the total calcium inorganic phosphorus surface tension and viscosity of the blood serum.

The mechanism of the diuresis which is produced in anesthetized cats and dogs by the intravenous administration of 50 mg of digitalis per kg is not dependent upon the production of quantitative changes in total calcium inorganic phosphorus, surface tension, or viscosity.

#### REFERENCES

- 1 Defendorf, J. H. Studies on the Bioassay of Digitalis. III. A New Diuretic Oligurie Cat Method, *J. Am. Pharm. A* 24: 369, 1935.
- 2 Clark, A. J. Influence of Ions Upon the Action of Digitalis, *Proc. Roy. Soc. Med. Pharm. Sec.* 5: 181, 1912.
- 3 Billinghamer, E. Über Wirkung und Zusammenhang von Calcium und Digitalis, *Klin. Wchnschr.* 8: 724, 1929.
- 4 Kevdin, N. Influence of Calcium on the Heart, Abstract, *J. A. M. A.* 87: 375, 1926.
- 5 Clark, E. P., and Collip, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.
- 6 von Berensky, G. Eine Studie über die Calcium und die Nebenschilddrüsenfrage, *Klin. Wchnschr.* 9: 1213, 1930.
- 7 Hermann, S. Neue Untersuchungen über den Kalkhaushalt, *Klin. Wchnschr.* 10: 1399, 1931.
- 8 Defendorf, J. H. The Effect of Hydrogen Ion Concentration Upon the Determination of Calcium in Blood Serum Phosphomolybdic Acid Centrifugates, *J. Lab. & Clin. Med.* 21: 65, 1935.
- 9 Fiske, C. H., and Subbarow, Y. The Colorimetric Determination of Phosphorus, *J. Biol. Chem.* 66: 375, 1925.
- 10 Du Nouy, P. L. An Apparatus for Measuring Surface Tension, *J. Gen. Physiol.* 1: 521, 1919.

## OBSERVATIONS ON THE ACTION OF CONGO RED ON NORMAL AND LEUCEMIC BLOOD\*

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THE following is an account of certain experiments undertaken to investigate the action of solutions of Congo red on blood cells and body fluids.

*Effect on Normal Blood.*—If a small quantity of fresh blood is added to an equal volume of 2 per cent Congo red solution in an unwaxed glass tube, the mixture remains fluid and a microscopic preparation shows that neither the red nor the white cells take up the stain, and also that the phenomenon of coagulation has not taken place. The blood cells remain uniformly dispersed, and if the coverslip is sealed down they will remain so for many hours. If the mixture is made by pricking the finger through a drop of the dye and mixing rapidly with a waxed glass rod even the platelets may be found to be discrete.

*Effect on Leucemic Blood.*—If blood from a case of myelogenous leucemia with a high white count is similarly treated and gently stirred, it quickly forms a thick, somewhat elastic gelatinous mass which lacks the firmness and solidity of a clot. With dark-field illumination and a high power objective no definite structure can be made out. The cells are arranged in rows or scattered diffusely throughout, but rouleau formation is not seen. The gel shows no obvious shrinking (syneresis) on standing and retains the dye for some time when suspended in water. When mixed with alcohol, it becomes granular and easily broken up, the dye then dissolving out readily into water.

If a similar specimen of leucemic blood is taken directly into the dye in a waxed capsule, so minimizing damage to the cells, the change does not occur for some time. The gel formation thus has a relation to the presence of ruptured cells similar to that obtaining in coagulation. It differs, however, in that the phenomenon can be produced by washed leucemic cells alone acting on the dye. On the other hand the serum from coagulated leucemic blood does not produce it, showing that the gel-forming substance is incorporated in the leucemic clot. Since cells of normal blood do not form the gel, it is clear that the gel arises from a dispersion through the dye of the contents of the primitive, easily ruptured, cells present in cases of leucemia.

This reaction can thus be used as a simple clinical test for the diagnosis of active leucemia, either myeloid or lymphoid.

Though this phenomenon occurs spontaneously with leucemic blood, it is not a specific reaction. It takes place because of the presence of many easily ruptured cells. This accounts for the fact that scrapings from most of the

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volume of 2 cc. In each of the tubes, 0.5 cc of a 5 per cent suspension of sheep red blood cells, serving as antigen, and a sufficient amount of inactivated rabbit serum which has been sensitized against sheep red blood cells, serving as amboceptor, are introduced. The tubes are incubated for thirty minutes at a temperature of 37° C. The rate at which hemolysis occurs at a given concentration of serum determines the amount of complement present in the patient's serum.

The figures obtained by us in normal people and patients suffering from different diseases, except rheumatic fever and other diseases which will be discussed separately, are fairly constant and range between 0.04 and 0.06 cc (Table I). They correspond to the figures reported by other authors. Table I includes one patient with subacute bacterial endocarditis superimposed on an old rheumatic heart lesion. During the typical course of the disease the complement titer was determined three times with the same result, namely, 0.05—which is normal.

TABLE I  
THE COMPLEMENT TITER IN VARIOUS CLINICAL CONDITIONS

NO	COMPLEMENT TITER	DIAGNOSIS
1	0.07	Hypertension Myocardial insufficiency
2	0.06	Pemphigus
3	0.08	Tuberculous peritonitis
4	0.06	Urticaria
5	0.04	Gastritis
6	0.04	Typhoid fever
7	0.05	Bronchial asthma
8	0.04	Tuberculous pleural effusion
9	0.04	Acute gastroenteritis
10	0.04	Catarrhal jaundice
11	0.04	Catarrhal jaundice
12	0.04	Typhoid fever
13	0.04	Pregnancy
14	0.04	Gastritis
15	0.05	Acute iritis
16	0.06	Acute dysentery
17	0.04	Bronchial asthma
18	0.06	Acute lymphatic leucemia
19	0.05	Sciatica
20	0.04	Asthma
21	0.08	Bronchial asthma
22	0.05	Chronic nephritis
23	0.07	Acute tonsillitis
24	0.05	Duodenal ulcer
25	0.04	Icterus Cholelithiasis
26	0.05	Chronic nephritis
27	0.05	Chronic tonsillitis
28	0.04	Myocardial insufficiency
29	0.05	Chronic nephritis
30	0.05	Nephritis Chronic tonsillitis
31	0.07	Sciatica
32	0.08	Sciatica
33	0.05	Subacute bacterial endocarditis
	0.06	Typhoid fever

Twenty five patients with rheumatic fever in active and inactive states were examined (Table II) with the uniform result of a definite lowering of the complement titer of the blood in the active stage of the disease, viz, the first attack or relapse with such manifestations of activity as fever, swelling of the joints, acute signs of cardiac involvement, and rapid sedimentation of the red

siderably, but as a rule, a fresh preparation of Grüber's stain will prevent coagulation if present in a total concentration of not less than 1 in 800. This was demonstrated in the following experiment: The dye in various dilutions from 1 in 50 to 1 in 400 was mixed in a capillary pipette in the proportion of one volume of dye to three of blood. The dye was drawn up to a mark first and the blood added directly from the finger, the mixture made on a slide, and incubated at 37° C. for fifteen minutes.

No coagulation took place when the total concentration of the dye was 1 in 800 or stronger.

*Effect on Serum Hemolysis and on Phagocytosis.*—It was shown by Klopstock<sup>1</sup> and by Gordon<sup>2</sup> that Congo red inhibited the hemolysis of sensitized red cells by complement. Gordon also found that the dye could be removed from the mixture by adsorption with carbon, thereby restoring the activity of the complement. Experiments similar to those of Gordon have given the same results, and an analogous inhibition was obtained when Congo red in a concentration of 0.5 per cent was added to a mixture of phagocytes, normal serum and bacteria. This inhibition was not due to any deleterious action on the phagocytes. Experiments showed that a saline suspension of cells could be exposed for twenty-four hours to a 1 in 200 concentration of the dye and, after several washings in saline, their phagocytic powers remained unimpaired.

The effect on the phagocytosis of bacteria sensitized with immune body was also tested. Antiserums were prepared in rabbits against *Staph. aureus*, *Staph. albus*, *Bact. coli commune*, and *Bact. coli communior*. After inactivation by heating for thirty minutes at 56° C., the serums were tested for their opsonic effect on the homologous bacteria, in the presence of the dye and also in its absence.

Phagocytosis was in all instances almost completely ablated by the dye, as in the case of normal unheated serum.

*Effect on Sensitization of Bacteria and Red Blood Corpuscles by Immune Serum.*—There are two processes concerned in phagocytosis due to an immune serum:

1. The sensitization of the organism by the specific serum.
2. The ingestion of the sensitized bacterium by a phagocyte.

The following experiment shows that the heat stable opsonin (immune body) becomes attached to bacteria in the presence of Congo red, and that it is the ingestion of bacteria by the cells that is prevented by the dispersing action of the dye. A suspension of *Staph. albus* in saline containing a 1 in 200 concentration of Congo red was sensitized with its homologous antiserum for thirty minutes at 37° C. A control suspension containing no dye was similarly treated. Both suspensions were washed in the centrifuge, resuspended in saline, and appropriate amounts added to washed leucocytes without the addition of any more serum.

The phagocytic index was practically the same in each case, showing that sensitization of the cells had taken place in the presence of Congo red. The inhibition of phagocytosis must then be due to prevention of the ingestion of the bacteria by the phagocytes.

age of infected sinuses, the temperature became normal and the joint manifestations practically disappeared. The sedimentation rate became slower, 60', while the complement titer remained at a low level.

In cases of rheumatic fever during the inactive stage, as exhibited by old rheumatic heart disease with or without heart failure and with no signs of activity, the amount of complement in the blood was found to be normal (Table II, B). This finding is of great interest, in view of the difficulty that sometimes arises in determining whether or not there is an activation of an old rheumatic process. The decrease of the complement titer will, according to our observations, disclose a relapse clinically not obvious.

In contrast to the findings in active rheumatic fever, the complement titer in patients suffering from rheumatoid arthritis, even in cases with the clinical signs resembling those of rheumatic fever, was within normal limits. These patients with rheumatoid arthritis had acutely inflamed joints, elevated temperature, and a rapid sedimentation rate of the red blood cells, but no clinical signs of cardiac involvement. The average age in these patients was higher than in the rheumatic fever group. In several patients the complement titer was determined on several occasions with practically the same results (Table III).

TABLE III  
THE COMPLEMENT TITER IN RHEUMATOID ARTHRITIS

NO	WCF	DATE	COMPLEMENT TITER
1	44		0.06
2	48		0.05
3	49		0.05
4	32		0.06
5	25		0.05
6	41	5/6	0.1
		6/27	0.04
		8/22	0.07
7	23		0.04
8	34	7/25	0.04
		8/22	0.05
9	52		0.05
10	27		0.06
11	22		0.06
12	35		0.04
13	44		0.06

The last group (Table IV) represents 7 patients suffering from an acute polyarthritis during a first attack or relapse. In this group the clinical differentiation between rheumatic fever and rheumatoid arthritis was not definite, the difficulty being in all cases, the uncertainty as to whether it was an attack of rheumatic fever without clinical signs of cardiac affection, or an acute form of rheumatoid arthritis. Some of these cases had a low complement titer.

#### DISCUSSION

This investigation, showing a definite decrease of complement in the blood of patients suffering from rheumatic fever in the active stage of the disease, finds partial confirmation in the work of Veil and Buchholz<sup>8</sup> and, later, Schrabel.<sup>9</sup> The former have determined the amount of complement in various

*Effect on the Migration of Cells.*—In a previous paper (Benians<sup>4</sup>) it was shown that, if a few cubic centimeters of a thick emulsion of gum tragacanth are injected into the subcutaneous tissues of a rabbit, leucocytes migrate into the mass within a few hours. The presence of bacteria in the "fixation area" increases the leucocytic invasion.

To test whether Congo red would affect this cell migration, the following experiment was carried out: 3 c.c. of a gum tragacanth emulsion containing 5 by  $10^9$  staphylococci per c.c. was inoculated subcutaneously into the flank of a rabbit. Into the other flank was injected a similar mass which had, however, been made up in a 1 in 200 concentration of Congo red instead of distilled water.

After twenty-four hours there was considerable swelling and edema on both sides. Each area was punctured, films were prepared from the exudate and examined microscopically. In the plain gum area, there was an abundance of cells mainly polymorphonuclear leucocytes, and many showed phagocytosis of the bacteria. On the Congo red side, on the other hand, there were only a few lymphocytes present, neither polymorphonuclears nor monocytes having invaded the gum. Many free bacteria were also seen and obtained in culture.

Since it has been shown in a previous paragraph that immersion in the dye solution for twenty-four hours did not harm leucocytes, one cannot suppose that these cells avoid the area because of any toxic effect from the Congo red. It would appear, therefore, that the dye overcomes the positive chemotaxis of the infected gum.

Benians<sup>5</sup> also noted that these subcutaneous fixation areas of gum would extract from the blood stream certain bacteria, particularly members of the coli typhoid group. This experiment was repeated with a Congo red gum to test whether bacteria are repelled in the same manner as leucocytes. Two rabbits were taken and one was inoculated subcutaneously in the flank with 4 c.c. of a plain sterile gum tragacanth emulsion. The other was given the same quantity of sterile gum made up in a 1 in 200 solution of Congo red. Six hours later each received 5 by  $10^9$  *Bact. coli* intravenously.

When the two areas were punctured twenty-four hours later, the plain gum was found to contain many cells and a considerable number of *Bact. coli*. In the Congo red gum, there were no cells but an abundance of bacteria could be seen. Cultures from both areas were positive but a much heavier growth was obtained in the Congo red exudate.

It is evident, therefore, that Congo red does not exert the repelling action on bacteria that it does on leucocytes.

After four days the plain gum area was an abscess cavity, containing many cells showing marked phagocytosis. Very few free bacteria were present and cultures gave only a few isolated colonies. The rabbit had wasted and appeared ill and was killed. Cultures from the heart blood, however, were sterile. The other rabbit which had received the Congo red gum was quite well and no abscess formed then or later. It is a curious paradox that the animal in which the bacterial infection had been apparently overcome should be in a worse state than the rabbit in which the bacteria were still multiplying freely.

plement according to Veil and Buchholz (streptococci or their products or a virus being the antigens) In contrast to this theory of rheumatic fever being an allergic disease, rheumatoid arthritis is considered, particularly by Cecil, Nicholls and Stainsby,<sup>17</sup> as a direct bacterial infection of the joints themselves According to these authors, who have based their opinion on bacteriologic studies of blood and joint fluid "rheumatoid arthritis is an infectious disease, caused in a high percentage of cases by a specific type of streptococcus which, after localization in a primary focus, is discharged from time to time into the blood stream and establishes metastatic infections in the joints" This conception is consistent with our finding of a normal amount of complement in the blood, as was found, also in other infectious diseases, such as typhoid, pneumonia, subacute bacterial endocarditis

The above conclusions are based on the very limited material available in Jerusalem, and it is hoped that similar investigations on larger numbers and a greater variety of cases will throw more light on this important question

#### SUMMARY

1 The amount of complement in the blood is diminished in the active stage of rheumatic fever, and normal in the inactive stage of the disease

2 The amount of complement is normal in patients suffering from rheumatoid arthritis with acute symptoms of the disease

#### REFERENCES

- Schlesinger, B. Signy, A. G., Ames, C. R. and Barnard, J. E. Etiology of Acute Rheumatism, Experimental Evidence of Virus as Causal Agent, *Lancet* 1 1145, 1935
- Cohn, A. E., and Swift, H. F. The Electrocardiographic Incidence of Myocardial Involvement in Rheumatic Fever, *J. Exper. Med.* 39 1, 1924
- Rothschild, M. A., Sacks, B. and Libman, E. The Disturbances of the Cardiac Mechanism in Subacute Bacterial Endocarditis and Rheumatic Fever. *Am. Heart J.* 2 256, 1927
- Master, A. M. and Jaffe, H. Rheumatoid (Infectious) Arthritis and Acute Rheumatic Fever, Differential Diagnosis, *J. A. M. A.* 98 881, 1932
- Cecil, Russell L. The Differential Diagnosis of Rheumatic Fever and Rheumatoid Arthritis. Libman Anniversary Volume, International Press, N. Y. 1 303, 1932
- Nicholls, E. E., and Stainsby, W. J. Streptococcal Agglutinins in Chronic Infectious Arthritis. *J. Clin. Investigation* 10 323, 1931
- Stuart Harris, C. H. A Study of Hemolytic Streptococcal Fibrinolysis in Chronic Arthritis, *Lancet*, p. 1456 1935
- Veil, W. H., and Buchholz, B. Der Komplementschwund im Blute. *Klin. Wchnschr.* No. 49 p. 2019, 1932
- Buchholz, B. Die Bedeutung der Komplementveränderung bei der rheumatischen Infektion. *Med. Welt* 7 1775 1933
- Schnabel, P. Komplementuntersuchungen bei rheumatischen Erkrankungen, *Med. Klin.* 29 714, 1933
- Schuetze, A. and Scheller, R. Experimentelle Beiträge zur Kenntnis der in normalen Serum vorkommenden globuloiden Substanzen. *Ztschr. f. Hyg.* No. 36, p. 270, 1901
- Bieling. Herdinfection und Immunität, *Verhandl. d. deutsch. Gesell. ch. f. inn. Med.*, Kong. 42 438 1930
- Paul, B., and Pely, M. Über die Abnahme des Blutkomplementgehaltes bei allergischen Krankheiten. *Klin. Wchnschr.* 14 163 1935
- Swift, H. F. Rheumatic Fever. Hektoen Lecture. *J. A. M. A.* 92 2071 1929
- Zinsser, H. and Yu, H. Bacteriology of Rheumatic Fever and Allergic Hypothesis, *Arch. Int. Med.* 42 301 1928
- Klinge, F. Über "Rheumatismus". *Klin. Wchnschr.* 9 580, 1930
- Klinge, F. and Fricke, G. Experimentelle Untersuchungen über anaphylaktische Entzündung der Gelenke, *Krankheitsforschung* 9 81, 1931
- Cecil, R. I., Nicholls, E. E., and Stainsby, W. J. Etiology of Rheumatoid Arthritis, *Am. J. M. Sc.* 181 12, 1931

## THE ELECTROPHORETIC MOBILITY OF HUMAN ERYTHROCYTES\*

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SINCE the electrophoretic mobility of erythrocytes is determined by certain surface characteristics of the cell, in order to determine what effect pathologic conditions may have on this surface, it is necessary first to know the average electrophoretic migration velocity of human erythrocytes. Several workers have recently reported investigations concerning the electrophoretic mobility of mammalian erythrocytes. Kosaka and Seki (1921) and Abramson (1929) have reported a difference in the migration velocity of the red blood cells of different mammals. The fact that race, sex and age have little influence on the zeta potential of the erythrocytes has been demonstrated by Abramson (1929). Although Schroeder (1927) has shown that the various Landsteiner groups have essentially the same electrophoretic migration velocity, we have included this phase in our investigation.

We have been unable to demonstrate a marked day to day variation in the migration velocity of the erythrocytes of a single individual. Although variation does occur, this variation is well within the limits of error and is not a factor which might alter the results.

### METHOD

As in our investigations on the electrophoretic migration of various microorganisms (1936), the migration velocity of the red blood cells was determined by means of the Kunitz modification of the Northrop-Kunitz microcataphoresis cell (1928), with a Bausch and Lomb 8 mm., 0.5 n.a., 21X objective and a 10X eye piece. Readings were made at two stationary levels, i.e., 0.21 and 0.79 of the inside depth of the cell. The observations were made immediately after the cell was filled. An applied potential of 97.5 volts, which gave a potential drop through the cell of 41.3 volts, was used. Twenty observations were recorded, ten with each polarity of the field. The average of these observations is the electrophoretic migration recorded. The migration velocities are expressed in terms of seconds per 0.5 mm.

The erythrocytes were collected from 222 medical students who were, apparently, in good health. The blood cell suspensions were made by adding 0.1 ml. of freshly drawn blood to 75 ml. of M/15 phosphate buffer solution of pH 7.4. The cells in this medium were negatively charged and migrated to the anode. Our observations agree with those of Abramson (1929) that the volume of blood added, within reasonable limits, does not interfere with the uniformity of the results obtained. Small clumps of cells migrated with the same speed

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cellular tissues of the body, which contain damaged cells, rubbed up with the dye give a similar result, and the same is true to a marked degree of purulent exudates

A portion of the gel, made from leucemic blood as described above, examined in a wet preparation shows that a few of the more primitive types, that is cells with relatively very large nuclei, have taken up the dye and the nucleus is deeply stained. The stained nucleus tends to expand and almost fill the cell, which may then rupture, the nuclear matter disappearing into the fluid, while the granules remain undispersed. Adult types of cell remain unstained and may show no change even after several days' exposure to the dye.

It is well known that Congo red is an acid dye, the colored anion being negatively charged. For the sake of comparison, therefore, the following similar experiment was carried out with an electropositive dye. Leucemic blood was added to a 1/200 solution of methyl violet 6 B (a strongly electropositive dye) and the results were exactly the reverse of those obtained with Congo red. The cells were grouped often massed together, both primitive and adult cells being deeply stained. In both types of cell the nuclei tended to be relatively small and concentrated into the center of the cell. In due course osmotic processes may occur and the cells rupture, but no gel is formed.

It is clear therefore that the electronegative or acid Congo red dye acts as a dispersing agent both of the cell units and their contents, and the basic or electropositive dye as an aggregating agent.

*Effect on Bone Marrow*—Wet preparations from the femur of a three month old guinea pig stained with Congo red showed that the large megakaryocytes as well as some others of the primitive myeloid cells almost always take up the stain. The supporting stroma cells also stain deeply and at once, but adult myeloid and lymphoid cells remain unstained.

Various explanations for the staining of these free primitive cells may be offered. They may be merely more fragile than the adult forms and be injured in the course of preparation. They may carry a lower negative charge than the adult cells and thus be less repellent to the electronegative dye. They may have a more permeable membrane and so allow the large molecules of dye to pass through. These three suggestions are automatically correlated in a physical sense and are what one might naturally expect of a young and growing cell.

The molecule of methyl violet which penetrates all cells rapidly is also very large but, perhaps on account of electropositive charge, it may agglomerate the substance of the cell membrane (thus producing a wider fenestration) in the same way that it aggregates the cell units and so render the membrane more permeable. The nuclear substance acts as an ampholyte to both of these powerful dyes and stains deeply once penetration is effected.

*Effect on Coagulation of Blood in Vitro*—When blood clots, the various elements appear to group themselves together in a definite sequence. First, the platelets come together, immediately the blood is shed, second, rouleau formation takes place, the larger surfaces of the red cells lying in apposition, and finally the protein particles form into threads. All these processes, as we have seen, are prevented by Congo red. Specimens of the dye vary con-

## SUMMARY

1. The electrophoretic migration velocity of the erythrocytes from 222 adult individuals was found to be 22.6 seconds with a calculated probable error of  $\pm 0.1$  seconds per 0.5 mm. with an applied voltage of 97.5 volts; 25.3 per cent of the cells studied were found to be within this range.

2. The migration velocity with the actual probable error was found to be  $22.6 \pm 0.5$  seconds and 86.6 per cent of the cells studied came within this range.

3. The electrophoretic migration velocity is a relatively constant factor of the erythrocytes of the adult individual. A significant day to day variation was not observed.

4. There is apparently no correlation between the migration velocity and the Landsteiner groups.

## REFERENCES

1. Abramson, H. A.: The Cataphoretic Velocity of Mammalian Red Blood Cells, *J. Gen. Physiol.* 12: 711, 1929.
2. Dozois, K. P.: Variations in the Electrophoretic Mobilities of *Escherichia*, *Aerobacter* and "Intermediate" Strains, *J. Bact.* 31: 211, 1936.
3. Kosaka and Seki: Communications of Akayama Medical Society, 1921.
4. Northrop, J. H., and Kunitz, M.: An Improved Type of Microscopic Electrophoretic Cell, *J. Gen. Physiol.* 7: 729, 1928.

## DOES ASPIRATION BIOPSY OF TUMORS CAUSE DISTANT METASTASIS?\*

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**Y**EARS must elapse before this question can be answered definitely by data from collected clinical cases. This experiment was carried out to secure information which might be obtained now, using the method of aspiration under the conditions given below.

Transplanted malignant tumors in mice and rats metastasize to the lungs and liver and other distant organs in a percentage of the cases. Our problem was to determine if this percentage would be increased by subjecting these transplanted tumors to the procedure of aspiration biopsy.

We should have preferred to use animals having spontaneous tumors, but we could not obtain a sufficient number of these. So we did these experiments on rats and mice into which we transplanted malignant tumors that we keep continually in vivo in the laboratory animals. The Flexner-Jobling rat carcinoma and mouse sarcoma 180 were selected. These tumors are widely used in laboratory work; and their characteristics and effect on the host are well known. We included in these results only animals in which the tumors grew progressively. Those animals that died considerably before the average for animals so treated were excluded from the figures.

The rat carcinoma implant attains a diameter of 1 cm. in two weeks; and death occurs usually after eight weeks when the diameter of the tumor is

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In a similar manner Bloom and Brown<sup>3</sup> showed that the attachment of hemolytic amboceptor to red cells is not prevented by the presence of multivalent anions although the hemolytic action of complement is inhibited. Gordon<sup>2</sup> also found that red cells can be sensitized and that bactericidal antibodies become fixed to bacteria in the presence of Congo red.

From the above experiments it appears that the inhibitory effect of Congo red is manifested in those processes which depend for their completion on the action of a heat labile (complementary) agent.

The following experiment was, therefore, carried out to test whether a similar body is necessary for the coagulation of blood. A small quantity of fresh blood was placed in a waxed tube and immediately centrifuged at high speed for half a minute. The supernatant plasma was drawn off into two capillary tubes and the ends sealed. One tube was placed at once into a water bath at 56° C for fifteen minutes and the second was kept at room temperature. Both tubes were then incubated at 37° C for a further fifteen minutes.

The unheated sample of plasma showed a clear jellylike contractile clot, but the heated one remained unclotted though slightly turbid.

It appears, therefore, that a heat labile body is essential in the coagulation of blood.

Repetition of Gordon's experiments on hemolysis have given substantially the same results though slight differences were found. In the present experiments red cells were sensitized with five minimal hemolytic doses of amboceptor in the presence of a 1 in 200 concentration of Congo red. The mixture was incubated for ten minutes in a water bath at 37° C. The cells were then washed three times with saline, made up to a 5 per cent suspension and tested by the addition of varying amounts of complement and further incubation at 37° C. A control series was included to which no Congo red had been added but which otherwise had received the same treatment.

The MHD of complement was approximately the same in the two cases, provided the reading was made after incubation at 37° C for one hour. It was found, however, that whereas hemolysis in the control series was complete in five minutes there was a long latent period in the case of the dye treated cells and lysis was only complete after about thirty minutes.

*Effect on Agglutination*—1 Standard serums (Group 3) for typing human blood were used in these experiments and red blood cells from a Group 2 individual. No inhibition of agglutination occurred when the reaction took place in the presence of concentrations of Congo red between 1 in 100 to 1 in 1,600. It was noted in passing that under the microscope the appearance of the agglutinated red cells is quite different from that seen in coagulation where the cells form rouleaux by the apposition of their larger surfaces, whereas in agglutination they adhere in irregular masses.

2 The action of Congo red on the agglutination of *Bact. paratyphosus B* by specific immune serum was also tested. The serum, at one fifth of its titer, was made up in concentrations of the dye of 1 in 400, 1 in 2,000, and 1 in 4,000. The mixtures were incubated at 56° C. No reduction of agglutination occurred as compared with normal controls of serum and bacterial suspensions in saline.

the beginning we aspirated both tumors of each animal by advancing the needle point in three directions within the capsule. This procedure was repeated on the second and on the third day thereafter. The animal was then allowed to live as long as it could without further tumor aspiration. The autopsies on these early experiments did not reveal any increase in the percentage of metastases in the aspirated animals over the nonaspirated control animals. We then commenced aspirating the tumors every day for as many as eight or ten successive days. And when the tumors had attained a 2 or 3 cm. diameter many more strokes than 3 were used in each aspirating period. Thus in the last 86 tumors aspirated the contents of the capsule were literally hashed. Due to the very cellular nature of these tumors, we were always able to secure a "positive" aspiration. A needle full of tumor material was yielded by our later forcible aspirations.

At death the aspirated animals were examined for metastases as were their controls. The contents of the pleural and abdominal cavities were examined with a magnifying glass for evidence of metastasis; and the lungs and liver were sliced throughout and so examined. Any tissue showing macroscopic evidence of a metastatic deposit was fixed in formalin and verified by microscopic examination. We lacked the technical services required for a serial section search for microscopic metastasis. We believe that these fast-growing malignant cellular growths would quickly attain a size easily visible to the eye during the weeks following aspiration if metastasis had occurred.

We do not report on regional metastasis in this work because we were forced to use transplanted tumor material. The original small implant may have some of its outer cells carried to regional nodes even before the residue which survives and "takes," forms its capsule and grows as a tumor. Double tumors, and sometimes a series of tumors, forming from a single implantation of tumor material are not uncommon, due to fragmentation of original implant. Thus regional metastases are excluded from the report.

In performing the aspiration we introduced the needle through healthy skin 1 or 2 cm. from the periphery of the tumor; and then advanced it subcutaneously up to and through the tumor capsule. The needle containing aspirated tumor material was withdrawn, of course, by the same route.

At autopsy, our first examination was directed to the subcutaneous area surrounding the tumor, that is, the area which was the site of the multiple needle routes. Careful dissection and inspection with a magnifying glass showed no evidence of implantation of tumor material along the needle track; and over 894 separate aspirations were done on the 126 tumors. The encapsulated tumor was then examined. The capsule showed no puncture defects, nor were any tumors found growing out from the transplanted tumor, through the capsule.

The tumor was then cut through its largest diameter and the cut surfaces examined for hemorrhage and effects of laceration due to the needle point. The usual central necrosis in the carcinomas precluded the forming of an opinion about this; but in the sarcomas we were surprised to find no remaining effects of the passage of the needle. If any had been present they had healed in the time elapsing between the date of the last aspiration and the death of the animal.

## DISCUSSION

One finds thus that Congo red prevents coagulation phagocytosis, and hemolysis but is without action on the sensitization of cells and the agglutination of red cells and bacteria. The inhibition is seen only in those processes in which a heat labile body takes part, and it seems reasonable to assume that the action is primarily concerned with this agent. Broom and Brown<sup>3</sup> attributed the inhibition of phagocytosis and hemolysis by potassium ferriocyanide to an increase of the negative electric charge of the cells by the multivalent anions of the salt. Brown (personal communication) has also found that small concentrations of Congo red increase the negative charge of human red cells and washed bacteria.

The interference by Congo red with the action of complement is associated, therefore, with an increase of the normal negative charge of the reacting cells and presumably of colloid particles, though it does not follow that the electrical aspect is the only factor to be considered. More generally one might say that Congo red exhibits a repelling or dispersing action in all these processes. Coagulation of blood and phagocytosis are obviously agglomeration effects which would be inhibited by dispersion of the reacting elements but, at first sight, it is difficult to consider hemolysis as a process of agglomeration rather than of dispersion. It must be remembered, however, that what has to be considered is the mechanism by which the large molecules of hemoglobin escape from the cell. It will then be realized that the essential change is in the cell membrane. If a grouping or agglomeration of the colloidal elements of the cell membrane occurred the interstices would be enlarged and the membrane become more porous. One cannot state definitely that such an agglomeration occurs in the membrane, but it may be significant that, observing under dark ground illumination the lysis of sensitized blood by complement, one notes the ghosts or cell envelopes grouped together, apparently intact and of normal size.

## SUMMARY

- 1 A solution of Congo red inhibits coagulation of blood, phagocytosis and hemolysis, but is without action on the sensitization of red cells and on red cell and bacterial agglutination
- 2 It is suggested that these effects can be explained by the dispersing action of the dye on colloid particles
- 3 When mixed with leucemic blood Congo red forms a gel This reaction can be used as a simple clinical test for active leucemic conditions

I am greatly indebted to Dr J C Broom of the Wellcome Bureau of Scientific Research for his help in coordinating these data

## REFERENCES

- 1 Klopstock, F Komplementadsorption durch Farbstoffe Biochem Ztschr 149 331, 1924
- 2 Gordon J The Action of Certain Dyes on Bactericidal Activity of Normal Serum and on Haemolytic Complement, J Path Bact 33 47, 1930
- 3 Broom J C, and Brown H C Further Observations on Electric Charge in Its Relation to Haemolysis and 1930
- 4 Benians T H C Furth on the Pathogenicity of Bacillus Coli in 5 123, 1924
- 5 Benians, T H C Septicaemia, The Selective Deposition of the Colon Typhoid Group of Bacteria in Fixation Abscesses, Brit J Exper Path 2 276, 1921

## FATTY INFILTRATION OF THE LIVER WITH HYPOGLYCEMIA\*

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**R**ECONSTRUCTION of the pathologic physiology from an autopsy makes the findings much more interesting and instructive. The following case illustrates the pathologic physiology of one and possibly two of the many functions of the liver.

### REPORT OF CASE

*History.*—A thirty-two-year-old American housewife was admitted to the hospital in a comatose state. She was found lying unconscious on the floor. She was sent to the hospital about five hours later. She had been a confirmed alcoholic for the past fifteen years. In addition to hard liquors she had been drinking beer for the past month. On the day of admission she had drunk about one quarter of a pint of hard liquor and had eaten very little food. Her husband stated that she developed black and blue areas easily, following minor injuries. There was no history of diabetes or of insulin injections.

*Physical Examination.*—The patient was uncooperative and semicomatose when the physical examination was made. The pupils were dilated, and the face appeared swollen. There was no clinical jaundice. The breath was sweet. The mucous membranes were blue. There were ecchymotic spots on the chest, the right flank, and on the right hip. The pulse could not be felt. The heart sounds were heard with great difficulty. The blood pressure could not be obtained in either arm.

The epigastrium was rigid. The liver was palpable 7 cm. below the costal border. The extremities were cold, cyanotic, and somewhat spastic. Reflexes were present and normal.

*Laboratory Findings.*—The red blood count was 4,350,000; hemoglobin 80 per cent; white blood count 13,000. The differential count showed normal percentages. The nonprotein nitrogen was 54.2 mg., and the blood sugar was 25 mg. per 100 c.c. The Hinton and Kahn tests were negative.

Intravenous 10 per cent glucose was given, but the patient died soon after it was started, five hours after admission.

*Autopsy.*—An autopsy was done eleven hours after death. For the sake of brevity only the positive findings will be given. The brain weighed 1,220 gm. The pia arachnoid showed marked edema. The liver weighed 2,050 gm. It was enlarged 7 cm. below the right costal border. The lower edge was rounded. It was yellow throughout. The anterior surface of the lower part of the right lobe was slightly granular. On section it cut with some difficulty. Frozen sections of the liver were stained with Sudan III. Paraffin sections were stained with hematoxylin and eosin. Examination of these sections showed that the cytoplasm of all liver cells was replaced with fat. The nuclei were round, located in the centers of the cells, and did not appear degenerated. The cells of the bile capillaries and the Kupfer cells appeared normal. The pancreas and adrenals, both of which influence the blood sugar level, were normal.

### DISCUSSION

*Glycogen Storage.*—Of the various functions of the liver, that pertaining to glycogen formation and storage concerns us here. In the liver glycogenesis and glycogenolysis are going on continuously. The actual deposition of glycogen

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as single cells. The electrophoretic migration velocity was obtained at room temperature, between 21° and 27° C.

Although most of the observations were made within a short time after the suspensions were prepared, in many instances the suspensions were kept for twelve to fourteen hours. We do not think that the suspensions may be held for as long as twenty-four hours at 37.5° C. or as long as forty-eight hours at 5° C. with no significant change in electrophoretic potential.

A frequent and careful comparison was made of the cell in question with a control blood. The blood of one of us was used as the control. A specimen of this blood was run through the apparatus (of course between the color determinations) and in case of a significant variation of the control the apparatus was completely checked and reset. When such variations did occur, they were traceable to faulty technique.

Within any one specimen a variation in the migration velocity of the erythrocytes was constantly found. When this variation exceeded  $\pm 0.2$  seconds from the average, another specimen was obtained and the work repeated. Of the twenty observations recorded most were within a limited range of velocity but a few were identical.

#### RESULTS

The mean electrophoretic migration velocity of the erythrocytes from 222 adult individuals was found to be  $22.6 \pm 0.1$  seconds per 0.5 mm with an applied voltage of 97.5 volts. Although the calculated probable error is but  $\pm 0.1$  seconds, in view of the various factors of technique the actual probable error is considerably greater and is about  $\pm 0.5$  seconds. Irrespective of the blood groups it has been found that 86.6 per cent of the cells studied were within the range of the actual probable error.

All attempts to correlate electrophoretic migration velocity with the Landsteiner groups proved futile. A short series of cross-agglutination tests has been made to determine whether or not individual bloods within a group that showed a wide variation in electrophoretic migration velocity could be matched. Within Group IV one specimen with a slow migration velocity failed to match one with a rapid velocity. This is the only instance in which this condition occurred. However, these results are not conclusive and more detailed work is necessary to determine any correlation of migration velocity with blood matching.

A measurable day to day variation was not obtained in the electrophoretic migration of the red blood cells from one individual. Frequent observations of velocity of the erythrocytes from the individual used as a control show a marked uniformity which continued over a period of months. This work was carried on over a period of some ten months during which at least 3 determinations were made on each of the 222 individuals observed. In no instance was a variation found that exceeded the actual probable error of velocity. The effects of various types of infection on the migration velocity of the erythrocytes will be the subject of further investigation.

of other liver functions. Other laboratory procedures such as blood urea and uric acid were not done because of oversight or the urgency of immediate treatment.

#### REFERENCES

1. Wiggers, C. J.: *Physiology in Health and Disease*, Philadelphia, 1935, Lea and Febiger, p. 884.
2. Gammon, G. D., and Tenery, W. C.: Hypoglycemia, *Arch. Int. Med.* 47: 829, 1931.
3. Rabinowitch: *J. Biol. Chem.* 83: 333, 1929.
4. Wiggers, C. J.: *Physiology in Health and Disease*, Philadelphia, 1935, Lea and Febiger, p. 925.
5. LeCount, E. R., and Singer, H. A.: Fat Replacement of the Glycogen in the Liver as a Cause of Death, *Arch. Path.* 1: 84, 1926.

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## EFFECT OF INTERMITTENT VENOUS OCCLUSION ON THE CIRCULATION OF THE EXTREMITIES\*

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### STUDIES OF SKIN TEMPERATURE .

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RECENT reports<sup>1, 2</sup> have indicated that intermittent venous occlusion is valuable in the treatment of chronic occlusive arterial disease. It was to determine the effects on the circulation, as indicated by changes in skin temperature, of intermittent venous obstruction that our studies were undertaken.

#### METHODS OF STUDY

The environmental temperature and humidity, the ingestion of food and water, nervousness, apprehension, sleep, exercise and other factors influence the temperature of the skin. For example, the temperature of the skin of the toes might increase if any procedure were carried out after breakfast, the increase resulting from the ingestion of food and not from the specific procedure. Determinations of the temperature of the skin for testing the effect on circulation of a procedure such as intermittent venous obstruction should be carried out only under conditions which eliminate as many as possible of the factors mentioned.

In our studies subjects who had not had food or water for at least six hours prior to beginning the study lay quietly in a room, the temperature and relative humidity of which were constant within one degree, for one hour before the study was begun. The intermittent venous occlusion was then carried out<sup>1, 2</sup> by placing a sphygmomanometer cuff about the leg above or below the knee and inflating it to a pressure of 30 to 90 mm. of mercury for two minutes, and then deflating it for two minutes. This alternate inflation and deflation was continued for periods as long as two hours. The temperature of the digits was measured with an electric thermometer, the thermocouples of which were placed on the skin of the terminal phalanges.

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about 2.5 cm. The mouse sarcoma tumor implant has a diameter of about 1 cm. in seven days, the animal lives about three weeks, when the tumor is about 2 cm. in diameter. Death occurs due to pressure necrosis of the overlying skin followed by infection and emaciation. The carcinoma is prone to central necrosis in the tumor, the sarcoma is not. All animals had the same diet and environment.

The animals bearing growing transplants were divided into two groups. The members of one group were subjected only to the usual course of the tumor implants. The tumors of the other animals were aspirated as described below. For each member of the aspiration group there was a member of the control group of the same strain, age and sex bearing the same tumor material implanted at the same time.

In the control group the 41 rats with carcinoma implants lived an average of fifty-eight days after tumor implantations. The 14 rats whose tumors were aspirated lived an average of fifty-four days. Fifty-one mice controls lived an average of twenty-five days after sarcoma implantations, as did 49 mice with aspirated tumors. Rats which died before the thirty-fifth day, and mice which died before the eighteenth day were excluded from the results, for the time interval was deemed too short for the growth of metastases.

There were 155 animals bearing 310 tumors which survived long enough to be included in this report. Of these 155 animals, 63 animals bearing 126 tumors were aspirated. We implanted a larger number of tumors than we did not aspirate in order to secure additional data on the percentage of occurrence of distant metastases in nonaspirated tumor-bearing animals.

The experiments were completed in a period of six months.

The technique of implantation is described in detail elsewhere.<sup>1</sup> In brief, the method is as follows: healthy animals bearing tumors which have "taken" and grown without regression, and in which the skin especially the skin overlying the tumor, shows no signs of infection or necrosis, are selected. The tumor is aseptically excised, divided by the scalpel, and a small piece, having a volume of the cube of 2 mm. is selected and sectioned from the growing peripheral portion. This material is inserted into the pointed end of a trocar. The trocar is introduced subcutaneously, with surgical technique, into the anterior abdominal wall, the point of the trocar is advanced cephaladward until it occupies a subcutaneous position between the bony chest wall and the skin. Then the obturator is advanced, causing the tumor material to lodge in this location, which is a considerable distance from the portal of entry of the trocar in the abdominal skin. Tumor material was implanted on both sides of the anterior chest wall.

Aspiration of the tumors was commenced when they had attained a diameter of about 1 cm. We desired to aspirate as soon as possible so that a possible metastatic deposit would have sufficient time to grow before the death of the animal occurred. However, the technique of aspirating these small tumors, in such lively animals, only lightly narcotized (to avoid anesthesia mortality), is difficult.

The aspirating of the tumors was done with a No. 18 gauge needle and a Record syringe according to the method described in detail elsewhere.<sup>2</sup> In

# LABORATORY METHODS

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## A PHOTOGRAPHIC METHOD FOR VISUALIZING THE SHAPE OF THE RED BLOOD CELLS\*

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THE shape of the erythrocyte is best seen in a diluted fresh preparation, as for instance in a counting chamber. Normally, the cells are remarkably uniform biconcave disks. In the anemias characterized by a congenital abnormality in the shape of the erythrocyte, spherocytes (congenital hemolytic icterus), sickloocytes (sickle-cell anemia), and ovalocytes (oval-cell anemia) occur either as the predominant cell or mixed with cells of normal shape. In sickle-cell anemia, bell-shaped corpuscles can be demonstrated also. Many variations of the biconcave disk are found, such as the flattened cell of obstructive jaundice and the microcyte of hypochromic anemia with a very thin center and elevated margin.

No simple method has been utilized for recording the shape of the red cell. While fitting together the positive and negative plates made by the Finlay color process, I found that the cells can be made to stand out in relief, thus giving a stereoscopic effect. To produce this effect the glass plates are matched with the emulsion side of both the positive and negative plate outside so as to give the impression of depth. Ordinary films cannot be used as the positive and negative images must be well separated. After the proper adjustment, the plates are fastened together with airplane cement and used for demonstration in a viewing box or for making prints (Fig. 1).

Since demonstrating this stereoscopic effect I have found that the same principle was used long ago with glass x-ray plates to show depth. The bas-relief commercial photographs are produced in the same manner. Photographs of different types of red cells are shown in Fig. 1. These illustrate the use and value of the procedure.

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The thoracic and abdominal organs were then examined in situ for gross evidence of metastasis then the lungs and liver removed and finely sliced. The surfaces of the slices were carefully examined with the magnifying glass. Tissue containing gross evidence of metastatic deposit was fixed in formalin and prepared for verification by microscopic examination.

Both the aspirated tumor bearing animal and its nonaspirated tumor bearing control were autopsied at the same time. As soon as one of this pair died the other was killed by ether. In a few cases we killed both when one or both became moribund.

The results given in Table I showed that 92 animals bearing 184 non aspirated tumors had 20 proved distant metastatic deposits while 63 animals bearing 126 tumors aspirated over 894 times showed 8 metastatic deposits.

TABLE I

RELATION OF REPEATED ASPIRATIONS OF RAT CARCINOMA AND MOUSE SARCOMA TO PRESENCE OF DISTANT METASTASIS AT DEATH

NO OF ANIMALS USED	TUMOR TISSUE USED	NUMBER OF ASPIRATIONS	DISTANT METASTASIS		
			MEDIASTINUM	LUNG	OTHER VISCERA
41	FRC	Controls	12	2	0
14	FRC	38 times	0	0	0
51	Sar 180	Controls	6	0	0
49	Sar 180	310 times	6	2	0

# CONCLUSIONS

Moderate or excessive aspiration biopsy procedure performed repeatedly on transplanted rat carcinoma and mouse sarcoma does not increase the percentage of distant metastases nor does it produce any demonstrable damage to the tumor capsule or result in implantation of the tumor along the needle tract.

# REFERENCES

1. Sugiura, K. and Benedict, S. R. The Influence of Certain Diets upon Tumor Susceptibility and Growth in Albino Rats. *J. Cancer Research* 5: 373, 1920.
2. Martin, H. E. and Ellis, E. B. Aspiration Biopsy, *Surg. Gynec. Obst.* 59: 578, 1934.

# DYES FOR ROMANOWSKY STAINS\*

## A REVIEW OF THE LITERATURE

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### 1. INTRODUCTION

IN THE forty-six years that have elapsed since Romanowsky stains first came into use, general understanding of the principles of the technique has progressed very little. Poor stains are often obtained with the same solutions that at other times yield entirely satisfactory preparations. Usually, one is at a complete loss about either the cause or remedy for such results because many unknown variable factors are acting simultaneously, and criteria for evaluating them have not been established.

Two closely connected reasons account for this state of knowledge about Romanowsky stains. First, the large amount of chemical investigation done on the dyes used has not come to the attention of sufficient numbers of hematologists. Second, experimental data on the factors concerned in obtaining a good stain have been lacking. While MacNeal's (1906 b, 1925) reviews are excellent, they, of course, omit recent developments as well as some points of special interest for a forthcoming paper on factors in Romanowsky staining. Conn's (1930, 1936) summaries are also of great value, but are very brief and based largely on MacNeal's. These are the only general reviews of the subject in English, although certain aspects are covered by other papers, such as those of Proescher and Krueger (1924) and Scott and French (1924 a, b). Indeed, nowhere in the literature is there a modern comprehensive treatment of this subject. The present publication represents an attempt to fill this need, leaving, however, certain technical points for future discussion.

### 2. CHEMICAL STUDY OF ROMANOWSKY STAINS

Chemical analysis of Romanowsky stains began with Malachowski (1891), who realized that some useful metachromatic dye was formed from methylene blue during the polychroming process. Opinions about the nature of this substance differed because of inadequate chemical knowledge. Unna (1891) initiated its isolation, separating it as a reddish dye soluble in ether, but Nocht (1898; 1899 a, b; 1901) studied it much more thoroughly. Not being able to identify it with certainty, Nocht did not commit himself and merely called the dye "Rot aus Methylenblau," because of its color in chloroform. The formation of this substance was then utilized as an indication of ripeness of polychromed methylene blue solutions.

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is the resultant of these two processes. Blood glucose is transformed into glycogen and is stored as such in the liver. Glycogen is formed by the liver from other substances such as 'galactose amino acids, lactic acid, dihydroxy acetone and possibly glycerol and fatty acids,' according to Wiggers.<sup>1</sup> The liver apparently possesses the regulatory power of storing or releasing glycogen in order to keep the blood glucose at a constant level.

Glycogen is also deposited in the muscles and in the skin. However, the glycogen of the muscles can be used only for muscular contraction and cannot, therefore, be used as a reserve supply when the liver is exhausted. The small amount in the skin is readily available for use by simple diffusion into the blood. It does not, however, serve as a reservoir for glycogen as does the liver.

*Hypoglycemia*—Hypoglycemia may result from (1) hyperinsulism<sup>2</sup> produced by a tumor or hyperplasia of the islets of the pancreas, (2) hypodermic injections of excessive amounts of insulin, (3) terminal diabetes, (4) alimentary hypoglycemia following ingestion of food, (5) loss of hormones which have a neutralizing effect or antagonistic action against insulin, e.g., in adrenal insufficiency, pituitary dysfunction, hypothyroidism or a combination of these, (6) renal diabetes, (7) lactation and pregnancy, (8) muscular dystrophy, (9) fatigue, (10) infections, (11) terminal hypoglycemia, and (12) destruction of the liver as in acute yellow atrophy,<sup>3</sup> also in extensive fatty infiltration of the liver following the toxic action of chloroform, phosphorus, arsenicals<sup>4</sup> or alcohol.

The case described above illustrates hypoglycemia with death following the destruction of the glycogen storage function of the liver by fat infiltration apparently due to the toxic effect of excessive alcoholic intake over a period of at least fifteen years. When the glycogen storage function of the liver is impaired fat infiltration is increased. The reason for this is not clear. One wonders whether or not the liver could have been restored in part to its normal function if the patient had survived this hypoglycemic crisis.

*Fibrinogen*—Another evidence of liver damage in this patient was the presence of ecchymotic spots on the chest, right hip, and right flank. These confirmed the husband's statement that she developed "black and blue" areas following minor injuries. It is generally accepted that fibrinogen is formed in the liver. With complete fat infiltration of the liver, fibrinogen manufacture would supposedly cease and consequently the blood fibrinogen would reach a low level.

There was no clinical evidence of loss of other liver functions. However, there would undoubtedly have been an increase in blood uric acid and a decrease in urea nitrogen if these tests had been done. No urine was secured to examine for leucine and tyrosine crystals.

#### SUMMARY

A case of hypoglycemia with death is described following complete fat infiltration of the liver in a thirty-two-year old female who was a confirmed alcoholic. A short time before death the blood sugar was 25 mg per 100 cc.

The cessation of the manufacture of fibrinogen by the liver was evidenced by ecchymotic spots on the trunk. There was no clinical evidence of the loss

though there is evidence that the orthoquinoid forms may be present also. It is evident that on each benzene ring, three hydrogen atoms have been replaced by other atoms or groups. Of the three replacing groups, two possess double bonds linking them to the same benzene ring. The terms para- and orthoquinoid refer to the relative positions of these two double bonds. Practically, the paraquinoid form indicates salt formation through a pentavalent nitrogen atom, while the orthoquinoid form indicates salt formation through a tetravalent sulphur atom. Most probably a tautomerism exists between the two forms (Hantzsch, 1906; Kehrmann and Schaposchnikoff, 1897; Kehrmann, 1906 a, b; Kehrmann, Havas, and Grandmougin, 1913 a, b; 1914; Pummerer and Gassner, 1913; Giemsa, 1922-1923; Clark, Cohen, and Gibbs, 1925; MacNeal and Killian, 1926).

Michaelis concluded that of the various substances present in polychromed methylene blue only methylene blue itself and methylene azure were useful in staining blood smears. He stated that methylene azure alone stained nuclei metachromatically, although eosin hastened this action, and methylene blue served as a contrast stain for the cytoplasm. His tests showed that polychromed methylene blue was composed mainly of these two useful basic dyes, with no methylene violet discernible. He, therefore, retained polychromed methylene blue as an easily available source of the necessary basic dyes, and advocated a stain similar to Nocht's, which consisted of aqueous solutions of eosin and polychromed methylene blue.

Giemsa (1902 a), under the direction of Nocht, became the first investigator to insist on stains made only with chemically purified basic dyes instead of with variable polychromed methylene blue solutions. While he agreed with Michaelis that methylene azure was important and methylene violet useless, he went farther in declaring that methylene violet was definitely detrimental. Contrary to Michaelis, he found it to be present in polychromed methylene blue. He explained Michaelis's failure to find this dye as being due to the fact that the latter had employed tests which were so inaccurate that as much as 40 per cent of methylene violet might be mixed with methylene azure and yet not be detected. Giemsa's experiments corroborated Bernthsen's results regarding the difficulty of controlling the decomposition of methylene blue by alkalis, so that in the polychroming process the simultaneous formation of the deleterious methylene violet together with the essential methylene azure could not be avoided. To exclude methylene violet, Giemsa, therefore, recommended the use of purified dyes instead of polychromed solutions.

Thus, although both Michaelis and Giemsa intended to employ pure methylene blue and azure together with eosin in their stains, only Giemsa achieved the desired result.

Continued research by Giemsa resulted in the discovery of an inexpensive way for preparing pure azure, which was then sold by the Grüber Company as azure I. A mixture of equal parts of azure I and methylene blue was called azure II. Giemsa's (1902 b) stain at first consisted of separate aqueous solutions of eosin and azure II which were mixed just before use to form eosin-azure II, the active stain. In 1904 the technique was simplified by dissolving

Nine patients without impaired circulation to the lower extremities were tested with the pneumatic cuff below the knee, pressure in the pneumatic cuff varying from 30 to 60 mm of mercury in the various studies. The average room temperature was  $24.6^{\circ}\text{C}$ , the average digital temperature  $29.4^{\circ}\text{C}$ , when intermittent venous occlusion was begun. At the end of an hour of the procedure recommended by Collens and Wilensky, the average skin temperature was  $29.7^{\circ}\text{C}$ , at the end of two hours it was  $28.8^{\circ}\text{C}$ . The lowest average skin temperature during the study was  $28.6^{\circ}\text{C}$ , the highest average skin temperature in the nine cases being  $30.5^{\circ}\text{C}$ . In a study of the effects of the procedure on the circulation of the toes of three patients with chronic occlusive arterial disease, the average temperature at the beginning of the studies was  $29.5^{\circ}\text{C}$ , the average room temperature being  $24.9^{\circ}\text{C}$ , and the average temperature after one and a half hours of treatment was  $30.2^{\circ}\text{C}$ .

In subsequent studies the pneumatic cuff was placed above the knee and pressures of 60 to 90 mm of mercury were used. In studies on five patients with hypertension or arthritis which were carried out for periods of one to two hours, there were increases of  $0.8^{\circ}\text{C}$ ,  $0.6^{\circ}\text{C}$  and decreases of  $3.2^{\circ}\text{C}$ ,  $0.5^{\circ}\text{C}$ , and  $0.2^{\circ}\text{C}$ , respectively. In studies of five patients with arteriosclerosis obliterans, for two hours the temperature increased  $0.9^{\circ}\text{C}$ , and  $0.2^{\circ}\text{C}$  and decreased  $0.7^{\circ}\text{C}$ ,  $0.9^{\circ}\text{C}$  and  $1.0^{\circ}\text{C}$ , respectively.

The results in individual studies did not show any consistent vasodilatation as evidenced by an increase in the temperature of the skin. Fluctuations from the basal temperature at the beginning of the experiments were minimal and probably those which would occur if intermittent venous compression had not been used. If the increases in temperature occasionally noted are to be accepted as evidence of vasodilatation resulting from intermittent venous occlusion, the decreases not uncommonly noted might perhaps be accepted as evidence of vasoconstriction induced by intermittent venous occlusion. It appears from our studies that intermittent venous occlusion does not cause vasodilatation under the circumstances of our studies.

#### SUMMARY

A study of the effects of intermittent venous occlusion on the skin temperatures of nineteen patients with or without occlusive arterial disease did not disclose evidence of significant or consistent vasodilatation resulting from the procedure.

#### REFERENCES

- 1 Collens W S, and Wilensky, N D. The Use of Intermittent Venous Compression in the Treatment of Peripheral Vascular Disease, Preliminary Report, *Am Heart J* 11 705, 1936.
- 2 Collens W S, and Wilensky, N D. The Treatment of Peripheral Obliterative Arterial Diseases by the Use of Intermittent Venous Occlusion. A Report of the Results in Twenty Nine Cases, *J A M A* 107 1960, 1936.

Second, Kehrman demonstrated that methylene blue yielded on oxidation (or polychroming) several azures, among which were the substances now called methylene azures A and B and symmetrical dimethyl thionin. He also synthesized them, thus proving what had been designated methylene azure in a loose sense to be asymmetrical dimethyl thionin. This he termed azure A, and he called trimethyl thionin azure B. Both of these are therefore demethylated oxidation products of methylene blue. The letters "A" and "B" are not logical with respect to the degree of demethylation.

However, Giemsa and Pappenheim (1912 a) did not agree that azure I and Kehrman's dimethyl thionin were identical. Even more than Giemsa, Pappenheim experimented considerably with the dyes necessary for Romanowsky staining. He (1901) was one of the first hematologists to use methylene azure, combining it with Ehrlich's triacid stain to obtain what appears to have been a Romanowsky stain. He (1904) observed also that the May-Grünwald stain could be changed to a Romanowsky stain by substituting for the methylene blue in it either methylene azure or still better, toluidine blue. Further study led Pappenheim (1911 a) to advocate a stain which was very unusual for that time, since it contained both acetone and methylene violet, as well as toluidine blue. These substances were used in combination with other constituents similar to those in Giemsa's stain, in Pappenheim's "Pan-chrom" solution. He modified the technique for its use later (1911 b, 1912 b). Pappenheim (1911 c, 1912 a) was also the first to propose a scientific type of stain to replace the empirical solutions of polychromed methylene blue. He made his solution with toluidine blue, methyl thionin (azure C?), methylene azure, and methylene violet.

At the instigation of the late Dr. G. Carl Huber, MacNeal (1906 a, b) undertook an investigation of this problem. He agreed with Giemsa that any stain utilizing polychromed methylene blue must necessarily be of varying composition because the polychroming process cannot be accurately controlled. From a chemical point of view Bernthsen and Kehrman also had found that factors of temperature, concentration, oxygen, and time, when varied slightly, resulted in significant differences in the final products. Hence, definite amounts of methylene azure and violet could not be obtained by polychroming methylene blue solutions. Wilson (1907), too, apparently independently, had come to similar conclusions.

After trying the various dyes available, MacNeal agreed with Unna rather than with Giemsa, that methylene violet was more important than was methylene azure. He, therefore, recommended a stain made with definite quantities of dyes: methylene blue, methylene violet, and eosin. Some of the controversial opinions of the time seemed due to use of admixtures of dyes, and MacNeal stated that further advances depended largely upon obtaining the dyes for study in a more highly purified state. Following subsequent investigations, he (1913, 1925) was able to develop adequate methods for obtaining such dyes of a greater degree of purity. After trying these, he became convinced that methylene azure was as important as methylene violet. Indeed, in the last stain proposed by him (1925), he used more azure than

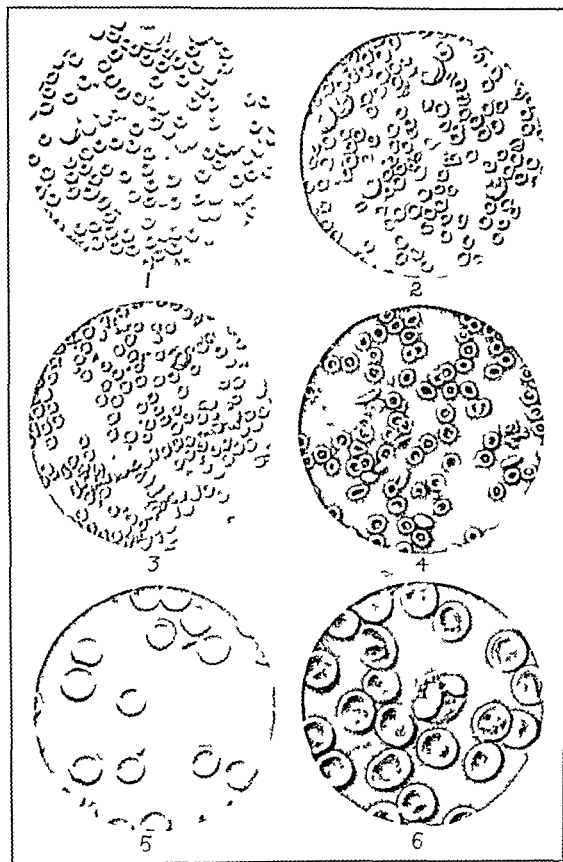


Fig 1—1 Normal red cells ( $\times 500$ ) 2 The typical shape of the red cells in a marked macrocytic and hypochromic anemia ( $\times 500$ ) 3 The spherocytes of congenital hemolytic icterus ( $\times 500$ ) Note the entire absence of the normal central depression 4 The red cells in sickle cell anemia ( $\times 500$ ) Note the small elevated area in the center of many cells which are in reality bell-shaped 5 A higher magnification ( $\times 1100$ ) of spherocytes (congenital hemolytic icterus) 6 The flattened cell of obstructive jaundice for comparison with the spherocyte

eosinates of these thiazin dyes; and with Snyder (1929) he investigated the rate of dealkylation (polychroming) of methylene blue in alkaline solutions. Many of these contributions were the direct result of the influence of the Biological Stain Commission.

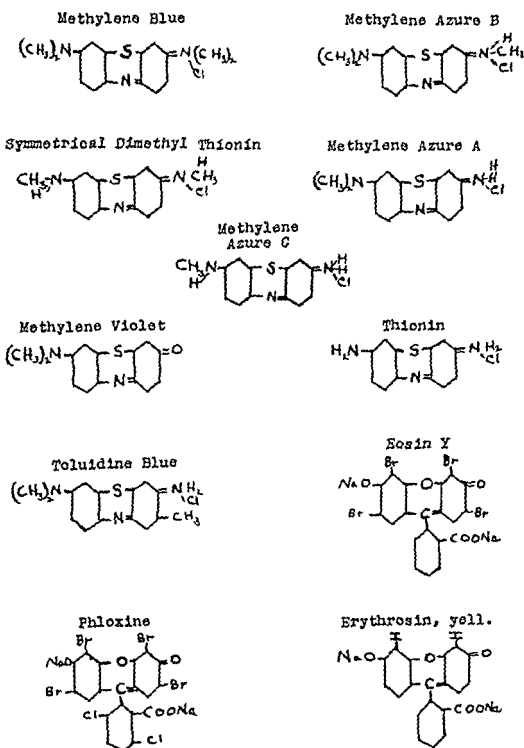
These valuable investigations, and especially MacNeal's outstanding results, did not seem known to European workers for several years. Baudisch and Unna (1919) and Unna (1922) did not mention them, and the first acknowledgment by Giemsa was made in 1934, when he took issue with MacNeal's initial (1906 b) formulas utilizing methylene violet for Romanowsky stains. A growing diffusion of knowledge of the recent chemical advances is indicated, however, by references given in later articles by Jerace (1934) and Lang (1935).

The purified thiazin dyes have been used also for stains other than those of the Romanowsky type. A list of all available publications in which they have been mentioned for any purpose whatsoever follows: French (1925, 1926), Giemsa (1902 to 1935), Geschickter (1930 a, b), Geschickter, Walker, Hjort, and Moulton (1931), Harris (1903), Haynes (1926 a, b; 1927; 1928), Hollande (1916, 1918), Holmes and French (1926), Jerace (1934), Jordan and Heather (1929), Kingsley 1935 a, b; 1936; 1937 a, b), Lang (1935), Laveran (1903), MacNeal (1906 to 1925), MacNeal and Otoni (1926), Marino (1904, 1905 a, b), May (1906), Pappenheim (1904; 1911 a, b, c; 1912 a, b), Richter (1927), Sacerdotti (1903), Scott and French (1924 a, b), Scott, Thompson, and Hydrick (1911), Strumia (1936), Tribondeau (1916 a, b; 1918 a, b), Viereck (1906), and Volkonsky (1928). A new use of azure II as a counterstain for nerve fibers has also been developed by Dr. J. A. Foley (unpublished).

On the other hand, Romanowsky effects have recently been obtained with dyes not previously used for such purposes (Epstein, 1922; Geschickter, 1930 a, b; Geschickter, Walker, Hjort, and Moulton, 1931; and Groat, 1936). Epstein used a polychromed toluidine blue solution which did not yield true Romanowsky effects but was quite similar except for the color of erythrocytes and eosinophilic granules. The researches of Geschickter and his coworkers made use of the eosinates of many different dyes derived from thionin. While Romanowsky effects were not reported, such effects probably were often obtained, on the basis of personal studies with thionin. Thus, in addition to toluidine blue used by Pappenheim and Epstein, thionin also is a dye which yields Romanowsky effects. Finally, the most recently published Romanowsky type of stain, Groat's (1936), uses not only thionin but also a dye heretofore not reported for such purposes, namely, methyl violet (C. I. No. 680).

The dyes just discussed are basic and are used in combination with eosin. But substitutes for eosin itself have also been reported. Rosin (1899) and Richter (1927) used erythrosin instead of eosin, while phloxine has been utilized by MacNeal and Otoni (1926), Haynes (1926 b), and Richter (1927). In analyzing the essential molecular grouping, Unna (1922) observed that resorcin can replace eosin, and he stated that Nocht had noticed this reaction previously. In personal experiments, erythrosin has been found capable of replacing eosin; but on the whole, results are not as satisfactory as with eosin.

Years previously, in 1885 and 1889 Bernthsen had published purely chemical studies of alkaline (silver oxide) oxidation products of methylene blue, a dye discovered by Caro in 1876. Bernthsen's solutions were similar to those used by hematologists and his method for polychroming was used later by Laveran (1899, 1900). In these studies methylene blue was found to be



- Kehrmann, F., Havas, E., and Grandmougin, E.: Über Farbbasen der Chinonimid-Farbstoffe, *Ibid.* 46, II: 2131, 1913 a.
- Kehrmann, F., Havas, E., and Grandmougin, E.: Zur Kenntniss der Farbsalze der Azin-Farbstoffe, II, *Ibid.* 46, III: 2802, 1913 b.
- Kehrmann, F., Havas, E., and Grandmougin, E.: Konstitution und Farbe der Azin-, Azoxin- und Thiazin-Farbstoffe. III. Mitteilung über Chinonimid-Farbstoffe, *Ibid.* 47, II: 1881, 1914.
- Kehrmann, F., and Schaposchnikoff, W.: Ueberführung des Phenyl-phenazoniums in Phenosafranin und des Isorosindulins von Nietzki und Otto in Naphtophenosafranin, *Ibid.* 30, II: 1565, 1897.
- Kingsley, D. M.: A New Hematological Stain, *Anat. Rec.* 61: suppl. 57, 1935 a.
- Kingsley, D. M.: A New Hematological Stain. I. Constituents and Methods of Use, *Stain Techn.* 10: 127, 1935 b.
- Kingsley, D. M.: Factors in the Control of Romanowsky Staining, *Anat. Rec.* 64: suppl. 27, 1936.
- Kingsley, D. M.: Acetone Solvents for Romanowsky Stains, *J. LAB. & CLIN. MED.* 22: 524, 1937 a.
- Kingsley, D. M.: Polychromed Methylene Blue as a Constituent of Romanowsky Stains, *Ibid.*, 22: 736, 1937 b.
- Lang, C. A.: Il fondamento delle colorazioni Romanowsky, Giemsa, e di quella May e Gruenwald, *Haematologica* 16: 795, 1935.
- Laveran, M. A.: Sur un procédé de coloration des noyaux des hématozoaires endoglobulaires des oiseaux, *Compt. rend. Soc. de biol.* 101: 249, 1899.
- Laveran, M. A.: Sur une méthode de coloration des noyaux applicable en particulier à l'étude des hématozoaires endoglobulaires, *Ibid.* 102: 549, 1900.
- Laveran, M. A.: Procédé de coloration des Protozoaires parasites du sang, *Ibid.* March 7, 1903. Ref. by Sabrazès in *Folia haemat.* 2: 250, 1905.
- Leishman, W. B.: A Simple and Rapid Method of Producing Romanowsky Staining in Malarial and Other Blood Films, *Brit. M. J.* 2: 757, 1901.
- MacNeal, W. J.: A Note on Methylene Violet as One of the Nuclear Dyes in the Romanowsky Stain, *Am. J. Anat.* 5: suppl. 1906 a.
- MacNeil, W. J.: Methylene Violet and Methylene Azure, *J. Infect. Dis.* 3: 412, 1906 b.
- MacNeal, W. J.: A Rapid and Simple Method of Staining Spirochaeta Pallida, *J. A. M. A.* 48: 609, 1907.
- MacNeal, W. J.: Tetrachrome Blood Stain: An Economical and Satisfactory Imitation of Leishman's Stain, *Ibid.* 78: 1122, 1922.
- MacNeal, W. J.: Methylene Violet and Methylene Azure A and B, *J. Infect. Dis.* 36: 538, 1925.
- MacNeal, W. J., and Killian, J. A.: Chemical Studies on Polychrome Methylene Blue, *Am. J. Path.* 1: 537, 1925.
- MacNeal, W. J., and Killian, J. A.: Chemical Studies on Polychrome Methylene Blue, *J. Am. Chem. Soc.* 48: 740, 1926.
- MacNeal, W. J., and Otoni, S.: Methylene Azure B in Staining Sections of Hematopoietic Tissues, *Am. J. Path.* 2: 478, 1926.
- MacNeal, W. J., and Schule: *Post-Grad.* 28: 982, 1913. Cited in MacNeal, 1922.
- Malachowski, E.: Zur Morphologie des Plasmodium malariae, *Centralbl. f. Klin. Med.* 12: 601, 1891.
- Marino, F.: Coloration des Protozoaires et observations sur la neutrophilie de leur noyau, *Ann. Inst. Pasteur* 18: 761, 1904.
- Marino, F.: Au sujet de la coloration des protozoaires. Réponse à l'article ci-dessus de M. G. Giemsa, *Ibid.* 19: 351, 1905 a.
- Marino, F.: Action des microbes vivant sur la solution de bleu azur dans l'alcool méthylique, *Ibid.* 19: 816, 1905 b.
- May, R.: Eine neue Methode der Romanowsky-Färbung, *München. med. Wehnschr.* 52: 358, 1906.
- May, R., und Grünwald, L.: Über Blutfärbungen, *Centralbl. f. inn. Med.* 23: 265, 1902.
- Michaelis, L.: Das Methylenblau und seine Zersetzungsprodukte, *Centralbl. f. Bakt., I. Abt.* 29: 763, 1901 a.
- Michaelis, L.: Bemerkung zu dem Aufsätze von Karl Reuter, *Ibid.* 30: 626, 1901 b.
- Nocht, Dr.: Zur Färbung der Malariaparasiten, *Ibid.* 24: 839, 1898.
- Nocht, Dr.: Nachtrag zu dem Aufsätze in No. 22: Zur Färbung der Malariaparasiten, *Ibid.* 25: 17, 1899 a.
- Nocht, Dr.: Zur Färbung der Malariaparasiten, *Ibid.* 25: 764, 1899 b.
- Nocht, Dr.: Discussion of Reuter's demonstration at Hamburg Med. Soc., June 18, München. med. Wehnschr. 48: 1261, 1901.
- Pappenheim, A.: Discussion of Reuter's demonstration at Hamburg Med. Soc., June 18, *Ibid.* 48: 1261, 1901 a.
- Pappenheim, A.: Eine panoptische Triazidfärbung, *Deutsche med. Wehnschr.* 27: 798, 1901 b.

azure II plus the compound dye azure II eosin in methyl alcohol and glycerin. This stock solution, diluted ten times with water before use, was obviously a more scientific stain than all previous ones since only known chemicals were used. Yet, Giemsa never published his method for preparing azure I, which therefore had to be purchased from Grubler. Nor did he reveal his method of making azure II-eosin, although he outlined a procedure to purify azure and stated the proper proportions of dyes required. Giemsa's failure to explain his methods fully has evoked disapproval by other investigators. Scott, Thompson and Hydrick for example remarked (1911 pp 320, 321) "To publish as he has done the results of a secret process in the guise of a scientific paper merits the severest condemnation."

At this period of development the chemical phases of hematologic technique were so little understood that May and Grunwald (1902) suggested a method almost identical with Jenner's (1899) which was then three years old. Actually, it is the same as Leishman's (1901) improvement of Jenner's technique. Since this was not a Romanowsky stain May several years later (1906) utilized current developments to improve the May Grunwald technique by following it with the use of methylene azure. This procedure was condemned by Viereck (1906) but it influenced Pappenheim (1906) to present a combination of the May Grunwald and the Giemsa stains. In the following years Pappenheim (1911 a, b, 1912 a) modified the details of its use several times. The May Grunwald Giemsa technique utilizes the eosinate of methylene blue in pure methyl alcohol (May Grunwald solution) for fixation. The solution is then diluted with water for staining and followed by the eosinates of methylene blue and methylene azure A in an alcohol glycerin water solvent (Giemsa's stain). This arrangement appears highly illogical and based on empiricism. Its main difference from Giemsa's stain would appear a priori to be merely any advantages that might accrue from longer fixation. Strumia (1936) very recently devised a single solution to replace the two separate ones, thus simplifying the technique.

Giemsa's opinions about the dyes necessary for Romanowsky staining were soon challenged by Harris (1903) and by Unna (1904). Harris observed that the eosinate of polychromed methylene blue was a better stain than Giemsa's solution. Unna's results were similar, and in explaining them he declared that the methylene violet present in polychromed methylene blue was necessary for good Romanowsky effects a conclusion directly contrary to Giemsa's views.

Meanwhile, chemists in Europe progressed farther in their study of the alkaline decomposition products of methylene blue. Important advances were made by Kehrmann (1906 a, b), in collaboration with Bernthsen. First, Kehrmann showed that methylene azure was not merely an oxidation product of methylene blue, with an  $-SO_2-$  group as Bernthsen had previously reported. Instead, Kehrmann proved that methylene azure was a simpler substance, containing fewer methyl groups than did the molecule of methylene blue (tetramethyl thionin). Bernthsen (1906) accepted this new evidence, and stated that Simon had presented data tending to a similar opinion in 1885.

# QUANTITATIVE DETERMINATION OF THE CONVULSIVE REACTIVITY BY ELECTRIC STIMULATION OF THE BRAIN WITH THE SKULL INTACT\*

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THE convulsive reactivity is usually measured on experimental animals by injecting convulsant toxins and determining the minimal convulsant dose. This method has certain disadvantages. Cumulative effects of the injected toxins may influence the results and must be avoided. If one wants to study the effect of anticonvulsant measures, one can hardly compare within a short time the convulsion thresholds before and after the application of this measure, because the amount of toxin in the animal's body consequent to the first injection may influence the threshold values at the next injection. If electric stimulation is used, these difficulties may be avoided. In this case, however, another problem arises. The reactive changes in the brain tissue and in the meninges following the exposure of the cerebral cortex may influence the results of a next experiment. Repeated daily measurements over a long period are prevented by these effects of exposure of the cortex.

It seemed, therefore, of interest to develop a method of electric stimulation with the skull intact. If one applies the electrodes to the skin over the skull, one has to overcome a much higher resistance than if one places the electrodes in the conjunctival sac, as was already observed by Jellinek<sup>1</sup> and Schilf.<sup>2</sup> The latter type of application was, therefore, chosen. An apparatus was built that allows the voltage as well as the duration of the stimulation to be varied. Since the resistance can easily be determined on a Wheatstone bridge, the convulsion threshold can be expressed in terms of electric energy.

The circuit of the apparatus is shown in Fig. 1. Alternating current from the 115 volt, 60 cycles line is used. The voltage is regulated by a Variac transformer (General Radio Comp. No. 200CU) which has the advantage that the output is essentially independent of the load; in addition, a voltmeter controls whether the desired voltage is obtained. The duration of the stimulation is varied in the following way. After leaving the Variac transformer, the alternating current is closed and opened by a relay, the magnet of which is activated by the discharge of condensers (from 1-20 $\mu$ F). The duration of the discharge and thus also the duration of activation of the magnet and of the flow of the alternating current is proportional to the capacity of the condenser, the resist-

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The method was shown at a joint meeting of the Philadelphia and the New York Neurological Societies, April 17, 1936, and at the Scientific Exhibit, American Medical Association, Kansas City, May 11 to 15, 1936. A somewhat similar method was described by T. Putnam, while this paper was in print (Science, May 28, 1937).

violet With these purer dyes available MacNeal (1925) was able to determine further that of the two azures A and B isolated by Kelmann (1906), azure A (asymmetrical dimethyl thionin) was the more useful in blood staining MacNeal's work thus culminated in a blood stain consisting of methylene blue, methylene azure A, methylene violet and eosin, and this has been sold since 1922 as MacNeal's tetrachrome blood stain (certified in 1925) The purified constituents methylene azure A (certified in 1925) and methylene violet (certified in 1927) have been on the market only for the past few years

However, improved methods of preparation developed by MacNeal and Kilian (1925, 1926) yielded crystalline thiazin dyes of still higher purity Use of these dyes led to a new conception of the value of azure B, and MacNeal and Otom (1926) then utilized it in a tissue technique Richter (1927) modified this, but he retained azure B as the important basic dye Giemsa (1934) recently stated that azure B might be more valuable than azure A as a stain, and he found that its eosinate yielded Romanowsky effects

It is quite apparent that MacNeal has removed blood stains from the category of empirical mixtures of such variable composition that manufacturers have not been able to prepare two successive identical batches, and put them, theoretically at least into the class of dyes whose action can be controlled His stain is used like Wright's and has been considered the most scientific blood stain yet proposed However, MacNeal's work has not received the recognition it deserves and his stain has therefore not been adopted widely

Indeed, only a few men have appreciated the importance of MacNeal's contributions Scott and French (1924 a, b) made interesting studies of polychromed methylene blue as well as of the purified dyes described by MacNeal and stressed the empiricism of using polychromed methylene blue solutions French (1925) applied MacNeal's work to the development of a Giemsa stain made with MacNeal's four dyes It is important to note that methylene violet was included On the basis of reactions reported by MacNeal, Holmes and French (1926) prepared highly demethylated oxidation products of methylene blue and described a closely related series of such substances They studied not only alkaline oxidation products but also acid ones Among them, they found a third azure, monomethylthionin, which they designated azure C This was then used in a tissue stain developed by French (1926) These chemical advances were applied by Haynes (1926 a, b, 1927, 1928) to histologic staining, with correlation of chemical structure and staining properties As progress continued, Holmes (1927) devised chemical tests for the presence of these dyes However, the method most often used is the spectrophotometric one While Rosin (1899) mentioned that the eosinate of methylene blue has a characteristic spectrum, it was Kelmann and his coworkers who utilized the absorption spectrum method most extensively in the study of the dyes discussed here Wilson (1907) was the first to apply it to blood stains This method is adequately explained by Conn (1936) Holmes found that azure B was a constant impurity present in methylene blue and that complete purification was not practicable Later (1929), he studied the formation of

## METHOD

One cubic centimeter of benzaldehyde reagent (2 gm. of paradimethylamino-benzaldehyde are dissolved in 50 c.c. of conc. HCl and then this solution is diluted with 50 c.c. of distilled water) is added to 9 c.c. of filtered urine. The colorimetric determination against the glass standard is carried out after five minutes. The following equations are used to determine the amount of urobilinogen in units in any definite amount of urine.

$$x = \frac{10}{R} \cdot \frac{v}{u} \cdot \frac{V}{100} = \frac{v \cdot V}{10 \cdot u \cdot R} = \frac{V}{u \cdot R}$$

$v$  = final volume of urine plus reagent (= 10 c.c.)

$V$  = total volume of urine

$u$  = volume of urine taken for the determination

$R$  = reading against the standard

It has been shown that there is a loss in the urobilinogen content of urines kept at room temperature. Schrumpf<sup>4</sup> has demonstrated that under the action of *B. coli* the nitrates of the urine are reduced to nitrites; the hereby liberated oxygen oxydizes the urobilinogen to urobilin. This loss in urobilinogen can be reduced to about 5 per cent, if the urine is put into the ice box immediately after voiding.

Normal urines contain no urobilinogen. In jaundice we have observed values from traces up to sixty and more units in twenty-four hours. The absolute daily values are of less importance for the evaluation of a case than the progression or regression of urobilinogen excretion. It is therefore essential to determine its daily excretion over a longer period of time. Such curves have been of great help in determining whether we were dealing with obstructive or parenchymatous jaundice.

## REFERENCES

1. Charnas, D.: Ueber die Darstellung, das Verhalten und die quantitative Bestimmung des reinen Urobilins und des Urobilinogens, *Biochem. Ztschr.* 20: 401, 1909.
- Adler, A.: Eine neue Methode der exakt-quantitativen Urobilinogen- (Mesobilirubinogen-) Bestimmung in Harn und Stuhl, *Deutsches Arch. f. klin. Med.* 154: 238, 1927.
- Terwen, A. J. L.: Ueber ein neues Verfahren zur quantitativen Urobilinbestimmung in Harn und Stuhl, *Deutsches Arch. f. klin. Med.* 149: 72, 1925.
2. Wallace, G. B., and Diamond, J. S.: The Significance of Urobilinogen in the Urine as a Test for Liver Function, *Arch. Int. Med.* 35: 698, 1925.
3. Abderhalden: *Handbuch d. biol. Arbeitsmethoden.* 1: 8, p. 341.
4. Schrumpf, A.: Einige weitere Urobilinogen- und Urobilinstudien, *Ztschr. f. d. ges. exper. Med.* 79: 564, 1931.

Thus, knowledge of the chemistry of these dyes has grown to the state where it can be applied to the solution of problems concerning the other factors which must be known in order to control Romanowsky stains (Kingsley, 1937 b)

## REFERENCES

- Baudisch, P, and Unna, P G Thiazinrot Dermat Wehnschr 68 49, 68, 81, and 97, 1914
- Bernthsen, A Studien in der Methylenblaugruppe Ann d Chemie 230 73, 1885
- Bernthsen, A Ueber den Eintritt von Schwefel in aromatische Paradinamine, die Constitution des Methylenroth, und neue Synthesen von Farbstoffen der Indamin und Thiodiphenylamingruppe, Ibid 251 1 1885
- Bernthsen, A Ueber die chemische Natur des Methylenazurs, Ber d deutsch chem Gesellsch 39, II 1804, 1906
- Clark, W M, Cohen, B, and Gibb, H D Studies on Oxidation Reduction VIII Methylene Blue, U S Publ Health Rep 40 1131 1925
- Conn, H J The Staining of Blood and Parasitic Protozoa, Stain Techn 5 127, 1930
- Conn, H J Biological Stains ed 3 196
- Fpstein, H Ueber eine neue Methode der Blutzellen- und Blutparasitenfärbung, Centralbl f Bakt 88 164, 1922
- French, R W Polychrome Stains I A Substitute for Giemsa's Stain, J Lab & Clin Med 11 352, 1925
- French, R W Azure C Tissue Stain, Stain Techn 1 79, 1926
- Geschickter, C F The Application of Dyes in the Cancer Problem, Ibid 5 49, 1930 a
- Geschickter, C F Fresh Tissue Diagnosis in the Operating Room, Ibid 5 81, 1930 b
- Geschickter, C F, Walker, E P Hjort, A M, and Moulton, C H A New Rapid Method for Tissue Diagnosis Ibid 6 3, 1931
- Giemsa, G Färbemethoden für Malaria-parasiten, Centralbl f Bakt 31 429, 1902 a
- Giemsa, G Färbemethoden für Malaria-parasiten, Ibid 32 307, 1902 b
- Giemsa, G Eine Vereinfachung und Vervollkommen meiner Methylenblau-Eosin-Färbemethode zur Färbung der Romanowsky-Nochtschen Chromatinfärbung, Ibid 37 308, 1904
- Giemsa, G Das Wesen der Giemsa-Färbung, Ibid 89 99, 1922 23
- Giemsa, G Geschichte, Theorie und Weiterentwicklung der Romanowsky-Färbung, Med Welt 8 1432, 1934
- Groat, W S A General Purpose Polychrome Blood Stain, J Lab & Clin Med 21 978, 1936
- Hantzsch, A Zur Natur der Oxazin- und Thiazin-Farbstoffe, Ber d deutsch chem Gesellsch 39, I 153, 1906
- Harris, H F A Modification of the Romanowsky Stain, Centralbl f Bakt 34 188, 1903
- Haynes, R Modification of the French Azure C Tissue Stain, Stain Techn 1 68, 1926 a
- Haynes, R Azure Stains, Ibid 1 106, 1926 b
- Haynes, R Investigation of Thiazin Dyes as Biological Stains I The Staining Properties of Thionin and Its Derivatives as Compared With Their Chemical Formulae, Ibid 2 8, 1927
- Haynes, R Investigation of Thiazin Dyes as Biological Stains II Influence of Buffered Solutions on Staining Properties, Ibid 3 131, 1928
- Hollande, A Ch Solution colorante a base d'eosinates d'azur et de violet de méthylène, Compt rend Soc de biol 79 746, 1916
- Hollande, A Ch Emploi de l'alcool amylique en technique histologique et plus particulièrement dans la méthode de Romanowsky, Ibid 81 223, 1918
- Holmes, W C Subsidiary Dyes in Methylene Blue, Stain Techn 2 71, 1927
- Holmes, W C The Chemical Analysis of Thiazin Eosinates, Ibid 4 49, 1929
- Holmes, W C, and French, R W The Oxidation Products of Methylene Blue, Ibid 1 17, 1926
- Holmes, W C, and Snyder, E F The Atmospheric Oxidation, or Dealkylation, of Aqueous Solutions of Methylene Blue, Ibid 4 7, 1929
- Jenner, L A New Preparation for Rapidly Fixing and Staining Blood, Lancet 1 370, 1899
- Jerace, F La colorazione dei parassiti malarici, Riv di malariol 13 114, 1934
- Jordan, J H, and Heather, A H Staining of Negri Bodies, Stain Techn 4 121, 1929
- Kehrmann, F Constitution der Thionin und Azovin-Farbstoffe, Ber d deutsch chem Gesellsch 39, I 914, 1906 a
- Kehrmann, F Ueber Methylenazur, Ibid 39, II 1403, 1906 b

Variable micro condenser  $C_1$ , variable resistor  $R_2$ , position of plate  $P$ , its size, size of the animal, sensitivity of the galvanometer and the inherent sensitivity of the circuit are the principal factors controlling the sensitivity of the method. Variable resistors  $R_3$  and  $R_4$  represent the gross and fine control of the zero shunt.

Tests conducted in which the galvanometer was placed one meter from the photokymograph, a plate of 40 sq. cm. was placed 1 cm. above a 350 gm. rat. The sensitivity could be controlled to such an extent that the recorded respiratory amplitude was just perceptible or had an excursion of 30 cm. A plate of 1 sq. cm. placed 0.5 cm. above the thorax of the rat was sufficiently large to allow for the recording of the respiration.

A record of rat respiration is shown with use of the larger plate and moderate sensitivity. The complete record also illustrates the stability of the circuit when oscillating, with the capacity constant and the circuit adjusted to moderate sensitivity.

In records obtained with this method the frequency of respiration can be determined. In addition, the relative differences between individual respiratory movements in regard to amplitude and the rapidity of the phases can readily be seen. Theoretically volumetric calibration is possible. The method should allow for important investigations upon the respiration of small animals where it is essential to have a means of recording such changes graphically and with sufficient amplitude.

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## A NEW METHOD OF RECORDING PHYSIOLOGIC ACTIVITIES\*

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### II. THE SIMULTANEOUS RECORDING OF MATERNAL RESPIRATION, INTRAUTERINE FETAL RESPIRATION AND UTERINE CONTRACTIONS

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A PREGNANT, near term, spinal animal is immobilized by securely tying to a special plate. The maternal animal, other than the head, is immersed in physiologic saline solution. This solution is maintained at body temperature by means of a water bath. A lower midline incision is made through the abdominal wall, the uterine segments delivered into removable troughs, and these in turn are immersed in the saline surrounding the mother. The troughs containing the uterus are filled with saline to a height that allows but a small portion of the uterus to project into the air. This exposed portion is moistened with saline at suitable intervals. The small fixed plate is placed over but not in contact with that portion of the uterus projecting into the air. Since capacity changes are recorded, the fluctuations of the fluid surrounding the maternal animal and the transmitted movement of the uterus will register as maternal respiration of reduced amplitude. The reduced amplitude is de-

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- Pappenheim, A Note on Hirschfeld's review of article by May and Grunwald (1902), *Folia haemat* 1 397, 1904
- Pappenheim, A Note on article of May (1906), *Ibid* 3 344, 1906
- Pappenheim, A „Panchrom“, eine Verbesserung der panchroptischen Universalfärbung für Blutpräparate jeder Art nebst Ausführungen über metachromatische Farbstoffe und die metachromatische Potenz des polychromen Methyleneblau (Unna), *Ibid* 11 194, 1911 a
- Pappenheim, A Über die Anwendung des polychromen May-Giemsa-Verfahrens zur Schnittfärbung, *Ibid* 11 227, 1911 b
- Pappenheim, A Einige Bemerkungen über Metachromasie gelegentlich des vorstehenden Artikels von S G Scott *Ibid* 12 325, 1911
- Pappenheim, A Noch einige Worte zur Azur-Romanowskyfrage, *Ibid* 13 187, 1912 a
- Pappenheim, A Zur Blutzellfärbung im dünnen Bluttrockenpräparat und zur histologischen Schnittpräparatfärbung bei hamatopoetischen Gewebe nach meinen Methoden, *Ibid* 13 339, 1912 b
- Proescher, F, and Krueger, A P A Simple and Rapid Method for the Preparation of Polychrome Methylene Blue and Thiazin Red A Rapid Method for Staining Frozen Sections With Thiazin Red, *J LAB & CLIN MED* 10 153, 1924
- Pummerer, R, and Giesner S Über die Desmotropie von panchroiden Salzen in der Thiazinreihe, *Ber d Deutsch chem Gesellsch* 46, II 2310, 1913
- Richter, M N A Modified Methylene Azure B Stain for Sections of Human Hemopoietic Organs, *Arch Path* 4 77 1927
- Rosin, H Ueber eine neue Gruppe von Anilinfarbstoffe, ihre Bedeutung für die Biochemie der Zelle und ihre Verwendbarkeit für die Gewebefärbung, *Berl klin Wchnschr* 36 251, 1899
- Sacerdotti, C Sugli eritrociti dei mammiferi colorabili a fresco con l'azzurro di metilene, *Arch per le scienze med*, 1905 Autorref in *Folia haemat* 1 89, 1904
- Scott, R E, and French, R W Standardization of Biological Stains, *Mil Surgeon* 55 229, 1924 a
- Scott, R E, and French, R W Standardization of Biological Stains II Methylene Blue, *Ibid* 55 337, 1924 b
- Scott, S G, Thompson, T O, and Hydrick, J L On Romanowsky Staining for Blood Cells, *Folia haemat* 12 302, 1911
- Strumia, M M A Rapid Universal Blood Stain May Grunwald Giemsa in One Solution, *J LAB & CLIN MED* 21 930 1936
- Tribondeau, L Procédé de coloration des liquides organiques et de leurs parasites, *Compt rend Soc de biol* 79 282, 1916 a
- Tribondeau, L Sur le mode d'emploi du bicosinate, *Ibid* 79 1022, 1916 b
- Tribondeau, L Coloration du sang à l'aide de deux colorants de préparation rapide et facile, genre May Grunwald et genre Giemsa, *Ibid* 81 639, 1918 a
- Tribondeau, L Coloration du sang à l'aide d'un colorant de préparation rapide et facile, genre bicosinate, *Ibid* 81 641, 1918 b
- Unna, P G Ueber die Reifung unserer Farbstoffe, *Ztschr f wissenschaftl Mikr* 8 475, 1891
- Unna, P G Die wirksamen Bestandteile der polychromen Methyleneblaufärbung und seine Verbesserungen, *Ztschr f prakt Dermat* 38 119, 1904
- Unna, P G Das polychrome Methyleneblau, *Ztschr f Bakt* 88 159, 1922
- Viereck, H Die polychrome Methyleneblaufärbung, *Ztschr f prakt Dermat* 52 1414, 1906
- Volkonsky, M S Die polychrome Methyleneblaufärbung, *Bull d'histol stain Techn* 4 96, 1929
- Wilson, T M On the Chemistry and Staining Properties of Certain Derivatives of the Methylene Blue Group When Combined With Eosin, *J Exper Med* 9 645, 1907

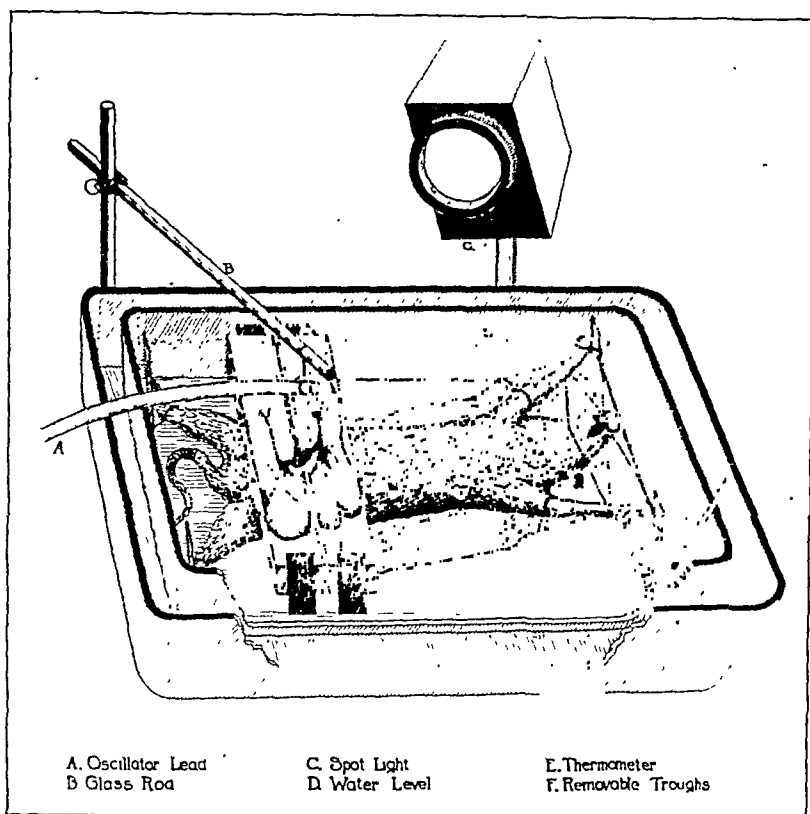


Fig. 2.—Illustration of the set-up, showing the details of the experimental pregnant preparation (rat).

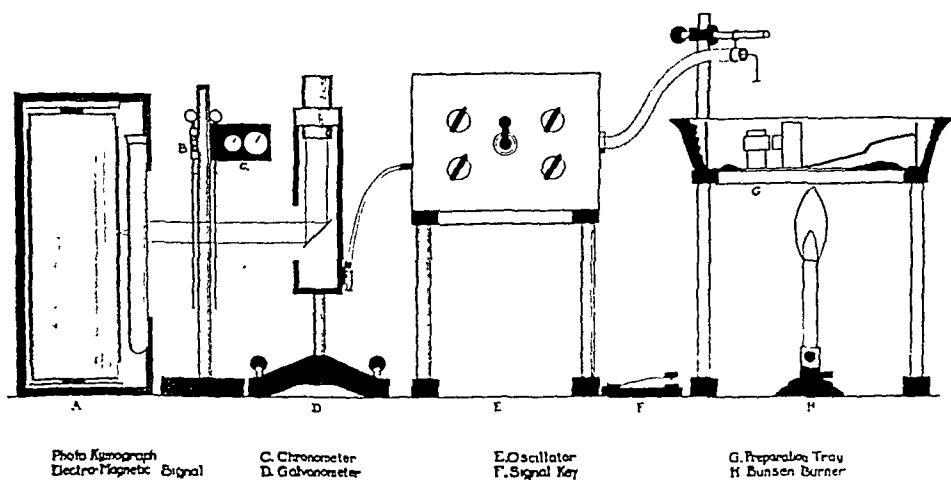


Fig. 3.—Illustration of the complete set-up.

ance of the relay circuit being constant\*. One must of course, watch the voltage of the dry cell battery that charges the condensers since a drop in this potential influences the duration of the condenser discharges. The duration of the discharge can easily be standardized by inserting into the circuit of the alternating current a signal magnet and projecting its shadow upon a fast photokymograph which has an exact time marker. With the arrangement used in these experiments, the relation between time in milliseconds ( $t$ ) and capacity in microfarads ( $c$ ) was  $t = 35c$ . Thus  $10\mu F$  correspond to  $35 \times 10$  milliseconds or 0.35 second.

Rabbits were chosen as experimental animals. Since the eyeballs lie on the side of the skull, the frontal lobes are much closer to the straight pathway of the current than in animals where the eyeballs are located in front of the skull. The electrodes are small, ovoid silver plates bent to fit the eyeballs. They are placed between the eyeball and the third lid. The upper and the lower lids are kept closed by small clamps. The animal is fastened on a short, elevated horizontal board by a band slung around its trunk while the head and the limbs remain free, so that motor reactions can easily be observed. In order to record these

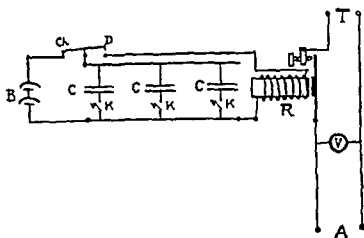


Fig. 1—T A Circuit of the alternating current (T, connection with the Variac transformer A connection with the animal V voltmeter) B, dry cell battery C condensers (1 2 5 10 20  $20\mu F$ ) K keys J relay Ch D, Key charging the condensers (position Ch) and discharging them (position D) into the relay

reactions, one may use one of the hind legs e.g., by connecting it with a lever that presses upon a Marey's tambour. The changes in pressure produced here are transmitted to a second tambour carrying the recording lever.

In order to determine the convulsion threshold, one sets the Variac at 15 volts and varies only the duration of the stimulation step by step starting with the smallest capacity and increasing it until epileptiform convulsions can be produced. If a duration of about one second proves ineffective, then the series of stimulations is started again at a higher voltage (20 volts) with the lowest capacitance. Usually 15 volts are sufficient. Between the single stimulations a pause of five minutes is intercalated. As long as the electric energy is below the threshold, one observes only defense reactions, such as running or struggling movements, that follow the stimulation immediately. If the threshold is reached,

\*The method reser in using condensers in c ever determines the lea voltage of a constant apparatus here describe is necessary to elicit convulsions

chronaxie (Lapicque Bourguignon) only he stimulation. The latter method how it which a voltage double the threshold excites a nerve or muscle. With the alternating current is determined which

sirable, because it allows for recording of fetal and uterine activity. The latter movements under these conditions are recorded with greater amplitude than the maternal respiration. This allows for ease in interpretation of the resultant record, and aids in the elimination of errors of interpretation in respect to transmitted maternal activity other than respiratory.

Uterine contractions are recorded directly in that the uterus undergoes relative displacement in respect to the plate when the contraction waves pass over the uterus. Fetal respiratory like movements<sup>1</sup> are recorded directly, in that displacements in the near term fetus result in similar displacement of the surrounding uterine wall. For the same reason other fetal activities are recorded. In addition, the fetal respiratory movements were checked by the observers viewing the fetus with the aid of a strong light transilluminating the uterus. The true fetal respiratory like movements as observed were recorded by means of the electromagnetic marker.

The procedure offers a new approach to the study of pregnant uterine and intrauterine fetal activity, and is characterized by the ease in which such activity is recorded and interpreted.

We wish to acknowledge the technical assistance supplied by J. W. Christensen and Gordon Newby.

#### REFERENCE

1. Rosenfeld and Snyder: *Proc. Soc. Exper. Biol. & Med.* 33: 576, 1936.

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## A MODIFICATION OF THE SILVER IMPREGNATION METHOD FOR THE STAINING OF RETICULAR LATTICED FIBERS\*

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WHILE various methods are available for the demonstration of argentosensitive reticular fibers, the results obtained are inconstant and the methods themselves somewhat complicated and time-consuming.

The following method has been found to be effective as well as simple in its technic and may be completed in a short time.

The tissue to be stained is fixed in anywhere from 1 to 10 per cent formalin solution for six hours or longer, good results having been obtained in specimens preserved in formalin for one year or longer.

1. Wash in running water for one or two minutes. If fixation has been prolonged, the washing should be continued for one to two hours.

2. Frozen sections (15 to 20 microns thick) are transferred from distilled water to 1:100,000-1:50,000 (0.001-0.002 per cent) potassium permanganate solution for two to thirty seconds, depending upon the character of the tissue and the duration of the fixation.

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3. Transfer to 20 per cent ammoniacal silver solution prepared by adding strong ammonia, drop by drop, to 20 per cent silver nitrate solution until the precipitate formed is just dissolved. Two or three drops in excess are added. The solution should have a perceptible odor of ammonia.

After immersion in this solution for two or three minutes,

4. Pass through distilled water to a freshly prepared 1 to 1.2 per cent formalin solution of pH 7 (6.5-6.9). During their immersion in this solution the sections should be kept in motion by means of a glass rod and the degree of silver impregnation controlled under the microscope.

5. Wash in water, dehydrate, and mount in Canada balsam.

If desired, the sections after silver impregnation may be placed in a weakly acid solution of gold chloride followed by fixation in 5 per cent hyposulphite solution and then thoroughly washed. Contrast stains (eosin, picric acid, etc.) may be used.

The following precautions are advisable: (1) The optimum duration of immersion in permanganate solution (two to thirty seconds) must be determined empirically for each preparation. (2) The silver solution must contain a slight excess of ammonia. (3) The formalin reducing solution should have a pH of nearly 7 and is best freshly prepared.

The method may also be applied to paraffin sections after complete removal of the paraffin.

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## STAINING METHOD FOR NASAL SMEARS FOR EOSINOPIHILE COUNTS\*

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IRENE WEIBER, OWOSSO, MICH.

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THE usual method of staining nasal smears as a blood film is with either Wright's or Giemsa's stain. This is not a good method, because it is very difficult to distinguish the neutrophiles from the eosinophiles even if the smear is very thin. If it is not thin, it is then impossible to distinguish them and the count is sure to be inaccurate. With this method the count can be done very rapidly as the eosinophiles stand out a bright pink on a blue background, while the neutrophiles are entirely blue.

### METHOD

Use undiluted Wright's stain for one minute. Dilute with distilled water until a metallic sheen appears on the surface, as in staining blood films. Allow to stain for four minutes. Wash in distilled water. Decolorize for two or three minutes with 95 per cent alcohol. Wash with distilled water. Counterstain for two minutes with Loeffler's alkaline methylene blue. Wash with distilled water and dry.

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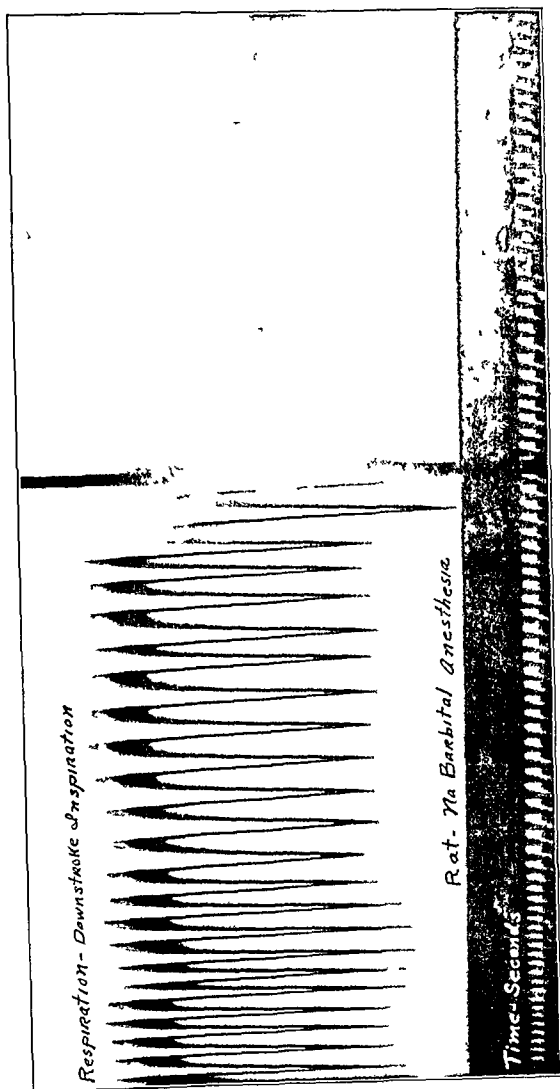


Fig 1 --Photokymogram 1 Simultaneous recording of 1 Maternal respiratory activity 2 uterine activity 3 Intrauterine fetal respiratory like move  
 2 Time in seconds C Electromagnetic marker

introduce an error which would be proportionately smaller the higher the phosphate concentration, and which might differ with different reagent lots, and with changes in room temperature and technique.

We, therefore, adopted the procedure of determining the apparent concentration of the blanks corresponding to each solution, including the standards, and applying these apparent concentrations as corrections. Thus, if the serum inorganic phosphate was to be determined, two sets of blanks were

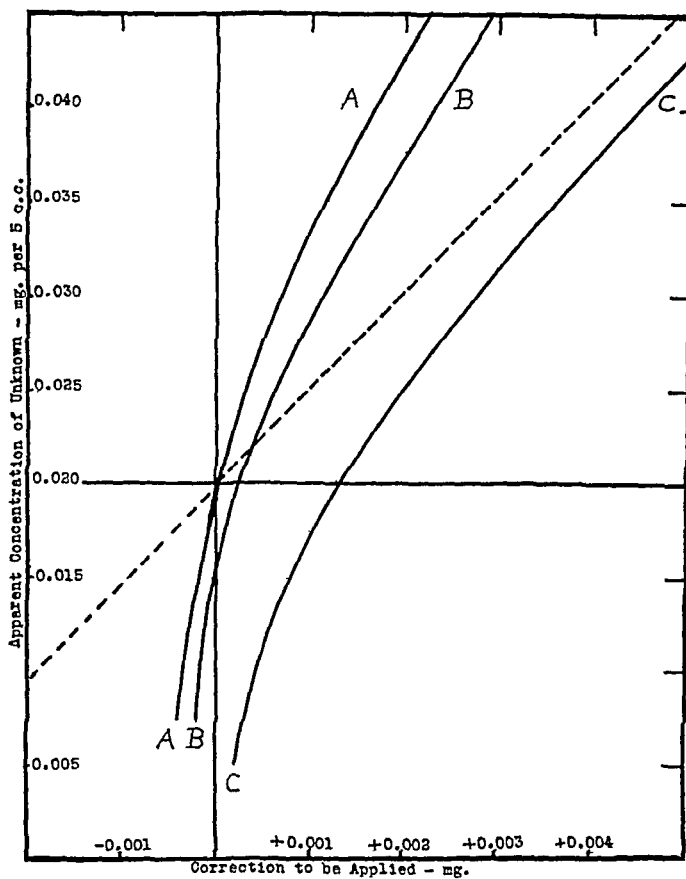


Fig. 1.

prepared. One, Bs, corresponding to the standard phosphate solution, contained 5 c.c. of water, 4 c.c. of molybdate reagent, and 1 c.c. of stannous chloride reagent. The other, Bp, corresponding to the unknown, contained 5 c.c. of 5 per cent trichloroacetic acid, 4 c.c. of molybdate reagent, and 1 c.c. of stannous chloride reagent. The blanks and the unknown solutions were then read in the colorimeter against the standard. Bs was then *added* to the known standard concentration to give the apparent concentration of the standard. The concentration of the unknown was calculated by comparison with this. Bp was then *subtracted* from the result. No correction for deviations

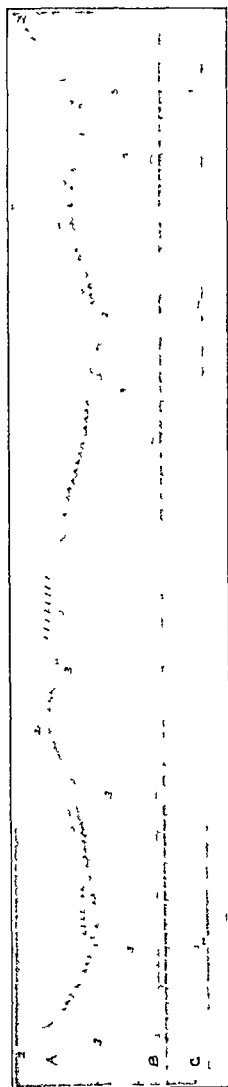


Fig. 1

factory determinations to be made in the presence of reagents contaminated by phosphate, or in very warm weather when blank solutions are likely to show considerable color even when the reagents are kept in the ice box.

Summary. Evidence is presented to show that a part of the deviation from Beer's law in phosphomolybdate solutions is due to blank error. Correction curves taking this into account have been prepared.

#### REFERENCE

1. Bodansky, A.: Phosphatase Studies. I. Determination of Inorganic Phosphate. Beer's Law and Interfering Substances in the Kuttner-Lichtenstein Method, *J. Biol. Chem.* 199: 197, 1932-33.

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## A SCIENTIFIC METHOD FOR THE PREPARATION OF NORMAL PHYSIOLOGIC SALINE SOLUTION IN HOSPITALS\*

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PHYSIOLOGIC saline solution is made in the majority of hospitals in an unscientific manner. In many institutions it is made by a student nurse, by dissolving a certain number of compressed tablets of sodium chloride in a liter of distilled water. If the wrong number of tablets is dissolved there is no means of determining the error. The solution is then filtered through paper or cotton, frequently requiring many refiltrations in order that a solution may be obtained entirely free of minute macroscopic particles. This product is then placed in an Erlenmeyer flask, stoppered with a gauze wrapped cotton plug and a yard or two of bandage is wrapped around the neck of the flask. Last, the flask is labeled with a strip of adhesive plaster on which is written in ink, more or less illegibly, "Saline Solution" and the preparation is autoclaved.

In the Graduate Hospital of the University of Pennsylvania production of physiologic saline solution was given to the laboratories by the Director of the Hospital, Dr. Donald C. Smelzer, and the following system was instituted with the resultant disappearance of complaints from the Hospital Staff relative to saline solution.

#### PERSONNEL AND EQUIPMENT

The personnel consists of two student technicians under the general supervision of the Hospital Chemist.

A room, approximately twelve feet square, is used for the preparation of saline solution and nothing else. It is equipped with counters on opposite sides, a rack for holding empty flasks, closets for storage and a large sink supplied with a combination hot and cold water faucet. A new Stokes water

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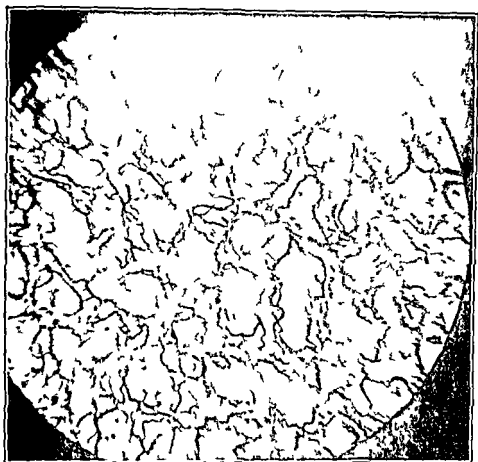


Fig. 1—The liver latticed fibers that have been ammoniacal silver impregnated with preliminary oxidation in  $\text{KMnO}_4$  solution. Average magnification.



Fig. 2—The spleen reticular fibers that have been ammoniacal silver impregnated with preliminary oxidation of the sections in  $\text{KMnO}_4$  solution. Small magnification.

The Standard saline solution, having been standardized and filtered, is bottled in Pyrex Erlenmeyer flasks. Each flask contains one-half its total capacity of liquid and is labeled with permanent baked-in letters "normal saline 0.85 per cent." The flasks are stoppered with a paper cap, the skirt or side of the cap completely covering the neck of the flask. The cap is held firmly around the neck by means of two wire loops that are tightened by twisting, using a hook with a screw handle for the operation. Each cap is stamped on the top with the date that the solution was made.

The flasks are then placed in large wire baskets capable of holding eight 2 liter flasks, each flask is in a separate compartment lined with lint. A steel truck, carrying eight baskets, conveys them from the laboratories to the sterilizers in another part of the Hospital.

#### PREPARATION OF STOCK SALINE SOLUTION

A 13.6 per cent solution of sodium chloride is prepared every Monday, using Merck's reagent sodium chloride and distilled water. Any solution remaining from the previous week is discarded. Ten liters are prepared by weighing 275 gm. of the sodium chloride, introducing it into a two liter volumetric flask, adding distilled water and when complete solution is obtained, sufficient distilled water is added to fill the flask to the graduation. This solution is poured into a five gallon Pyrex glass carboy and the procedure is repeated five times, yielding the desired ten liters. This solution is called Stock saline solution. It is now mixed by drawing air through it, as described above. Its exact sodium chloride content is determined by means of a chemical titration which will check any error in weighing or dilution.

Several solutions are required for the titration. These are called Stock solutions, to differentiate them from a group of similar solutions but of weaker concentration used in titrating the 0.85 per cent saline solution.

*Stock Silver Nitrate Solution.*—Using an analytical balance, 39.529 gm. of silver nitrate, Merck's reagent, is weighed and then transferred to a 100 c.c. glass stoppered volumetric flask. Distilled water is added and when solution is obtained, sufficient distilled water is added to fill the flask to the graduation. The contents are mixed and transferred to a brown glass stoppered bottle and kept in a dark closet to protect it from light.

*Stock Ammonium Thiocyanate Solution.*—One hundred and eighty grams of ammonium thiocyanate are dissolved in water in a glass stoppered cylinder and diluted to a volume of 1 liter. This solution must be standardized against the Stock silver nitrate solution, so that one volume of it will be equivalent to one volume of the silver solution. This is accomplished by pipetting 15 c.c. of Stock silver nitrate solution into a 250 c.c. Erlenmeyer flask, to which is added 15 c.c. of concentrated nitric acid, 0.3 gm. of powdered ferric ammonium sulphate and approximately 30 c.c. of water. A 25 c.c. burette is filled with the Stock ammonium thiocyanate solution, and it is slowly titrated into the Erlenmeyer flask until a salmon pink end point is obtained that will persist for fifteen seconds. A reading of the burette is taken and if 15 c.c. were used, the Stock ammonium thiocyanate is correct. The contents of the flask are dis-

## A MODIFICATION OF THE BODANSKY METHOD FOR THE DETERMINATION OF INORGANIC PHOSPHATE\*

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IN 1932 Bodansky<sup>1</sup> published a modification of the Kuttner Lichtenstein method for determining inorganic phosphate. He included corrections for deviations from Beer's law in the absence of interfering substances, and for the interfering substances which are present when the method is used for phosphatase determinations. The method consists in adding to 5 cc of unknown phosphate solution 4 cc of a solution of sodium molybdate in sulphuric acid, followed by 1 cc of a solution of stannous chloride in hydrochloric acid, and comparing the depth of color of the resulting blue phosphomolybdate with that formed by a standard phosphate solution under the same conditions. When the method is used for the measurement of serum phosphatase, the determination of serum inorganic phosphate is made in a solution containing 5 per cent trichloroacetic acid, while that of phosphatase is made in a solution containing 5 per cent trichloroacetic acid + 0.21 per cent sodium barbiturate + 0.25 per cent sodium beta glycerophosphate. The concentration of the unknown solutions is calculated by means of a table showing deviations from Beer's law, and a correction is then applied for interfering substances. The author states that, if the molybdate and stannous chloride reagents are kept cold, then the blanks prepared by mixing the reagents with 5 per cent trichloroacetic acid are practically colorless.

In the use of this method, we encountered difficulty in obtaining colorless blanks, even solutions which appeared only faintly colored to gross inspection giving significant apparent phosphorus concentrations when read against standards in the colorimeter. The depth of color varied greatly from day to day, but the average apparent phosphorus content of one hundred solutions containing 5 cc of water plus color reagents was 0.0039 mg, of solutions containing 5 cc 5 per cent trichloroacetic acid plus reagents was 0.0022 mg, and of solutions containing trichloroacetic acid, glycerophosphate, and buffer plus color reagents was 0.0030 mg. The only occasions upon which the blanks were free of appreciable color were when the stannous chloride solution had deteriorated sufficiently to cause gross error in known phosphate determinations. While the error in reading such very faint colors of course is large, it was found that, when solutions known to contain 0.0020 to 0.0040 mg of phosphorus were made up, the apparent concentrations of the blanks and of the known phosphorus solutions were approximately additive. It therefore seemed reasonable to suppose that an excess color equal to that of the appropriate blank was present in a determination. This excess would

\*From Memorial Hospital.

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$\frac{\text{Volume Stock Saline Sol.}}{10} \times (\text{Titer} - 5) \times 0.136 \approx \text{gm. of sodium chloride to be added to the Stock saline solution.}$

If the volume of Stock saline solution is 9,980 c.c. the formula is

$$\frac{9980}{10} \times (6-5) \times 0.136 = 135.728 \text{ gm. sodium chloride to be added.}$$

After addition of the sodium chloride, the solution is mixed and titrated.

The Stock saline solution, being of the correct percentage strength, is filtered through the sintered glass filters as described above and is then ready for use.

#### PREPARATION OF STANDARD SALINE SOLUTION

One liter of Stock saline solution is measured in a liter volumetric flask and introduced into a 5 gallon Pyrex glass carboy, to which is added 15 liters of fresh distilled water measured by means of a three liter volumetric flask. It is mixed by drawing air through it for a period of twenty minutes. This solution is normal saline solution. To be certain that the normal saline solution is 0.85 per cent and that there has been no error in the measuring, it is titrated with reagents similar to the ones used in titrating the Stock saline solution, except that these reagents are weaker in concentration and are called "Standard" solutions to distinguish them from the "Stock" solutions mentioned above.

*Standard Silver Nitrate Solution.*—Two and forty-seven hundredths grams of silver nitrate, Merck's Reagent, are weighed on an analytical balance and then dissolved in distilled water in a 100 c.c. volumetric flask. When all the chemical is dissolved, distilled water is added to the graduation on the flask and the solution mixed. It is then transferred to a glass stoppered brown bottle and kept in a dark closet. One cubic centimeter of this solution is equivalent to 1 c.c. of 0.85 per cent sodium chloride solution.

*Standard Ammonium Thiocyanate Solution.*—Eleven and twenty-five hundredths grams of ammonium thiocyanate, Merck's Reagent, are dissolved in distilled water and diluted to a volume of one liter. This solution must be standardized against the Standard silver nitrate solution in the same manner that the Stock ammonium thiocyanate solution was standardized against the Stock silver nitrate solution, i.e., 15 c.c. of Standard silver nitrate solution are pipetted into a 250 c.c. Erlenmeyer flask, to which is added 15 c.c. of concentrated nitric acid and 0.3 gm. of powdered ferric ammonium sulphate. About 15 c.c. of distilled water are added, the flask is shaken and kept in a dark place for five minutes. A 25 c.c. burette is filled with the Standard ammonium thiocyanate solution and is titrated into the flask until a salmon pink end point is reached. If the reading on the burette is 15 c.c., then the thiocyanate solution is correct and 1 c.c. of Standard ammonium thiocyanate solution is equivalent to 1 c.c. of Standard silver nitrate solution. The contents of the flask are discarded. If less than 15 c.c. of the thiocyanate solution were used, it is strong in concentration and must be diluted with distilled water according to the formula:

from Beer's law was introduced at this point, as this was included in the curve for corrections for interfering substances described below

With this method it was not possible to use Bodansky's tables for deviation from Beer's law and for interfering substances. New correction curves were therefore prepared and are given in Fig 1. All concentrations were read against the average of two standards containing 0.0200 mg. phosphorus. The average difference between the two standards was about 1 per cent. Each curve represents 95 to 110 determinations made in the course of 20 to 25 experiments. The average number of readings made against a single pair of standards was thus only 4 to 5, so that possible errors in one or two standard preparations could not cause important errors in the curves. In the curves, the apparent concentration of the "unknown" obtained by comparison with the apparent concentration of the standard and subtraction of the appropriate blank is plotted against the correction to be applied. The dotted line shows Bodansky's data for deviations from Beer's law plotted in the same way.

Curve A shows corrections for known phosphate solutions in water.

Curve B shows corrections for known phosphate solutions in 5 per cent trichloroacetic acid, as for determination of serum inorganic phosphorus.

Curve C shows corrections for known phosphate solutions in 5 per cent trichloroacetic acid + 0.21 per cent sodium barbiturate + 0.25 per cent sodium beta glycerophosphate, as for determination of phosphatase.

A correction curve was also prepared for phosphate solutions containing 5 per cent trichloroacetic acid + 0.21 per cent sodium barbiturate. This was found to coincide with Curve B, showing that sodium barbiturate did not interfere with the development of the phosphomolybdate color. The correction curve for a saturated solution of calcium hexose diphosphate in sodium barbiturate and trichloroacetic acid was nearly the same as Curve C. Curve A was the same whether the reagents were used at room or ice box temperature, although the blanks were much larger with the warmer solutions. It was found that sodium fluoride, when present in a concentration of 0.04 per cent (M/100) did not affect Curve A. On the other hand, 0.2 per cent potassium oxalate reduced the color intensity by about 8 per cent.

Curve A, which is not used in actual calculation of experimental data, represents deviations from Beer's law in the absence of blank error. The deviations are much smaller than those reported by Bodansky, the differences being in the same sense that they would be had his determinations contained uncorrected blank errors.

Curves B and C embody corrections both for deviations from Beer's law and for the interference of trichloroacetic acid and substrate with the development of the phosphomolybdate color. These corrections also are smaller than those reported by Bodansky.

Calculations made by the method presented here are more time-consuming than those made by means of Bodansky's tables. Sixty-seven successive phosphatase determinations were calculated by both methods with an average difference of only 2 per cent, but with a difference for individual experiments up to 10 per cent. The present method thus presents no advantages for ordinary clinical work under ideal conditions. It does, however, allow satis-

## FINISHED PRODUCT

We now have a sodium chloride solution actually 0.85 per cent in strength, checked by chemical titration. It is perfectly clear, having been filtered through porous glass, eliminating any possibility of minute macroscopic particles from filter paper. It is bottled in flasks having a permanent label, saving the cost of time and labor in constantly renewing adhesive plaster labels which are also hard to read. The paper cup gives the date of manufacture of the solution, takes a few seconds to apply to the flask and cannot be removed without unscrewing the small wire loop. The cap and wire cost less than one cent. The paper cap will keep a sterile solution free from contamination, experiments having proved that culture media can be kept sterile in paper capped flasks for over six months. The average time required for preparing 32 liters of saline solution from the start to the capped product is approximately three hours.

## CONCLUSION

A method is offered that is safe, efficient and economical for the manufacture of normal physiologic saline solution.

The author wishes to acknowledge the valuable aid of his assistant, Mr. Duncan E. King, in organizing this department of the laboratories.

still, steam heated, capacity ten gallons of distilled water per hour, is located in one corner of the counters.

#### GENERAL PLAN OF THE SYSTEM

The principle of the method consists in the preparation and standardization, by means of chemical titration of a 13.6 per cent solution of sodium chloride which is made once a week and is known as the Stock saline solution. This solution is diluted each day with fresh distilled water, 1 liter of it to 15 liters of the water, thereby making a 0.85 per cent solution of sodium chloride known as Standard saline solution. This Standard saline solution is also titrated to determine its percentage of sodium chloride, thereby checking any error that might have been made in diluting the Stock saline solution. Both

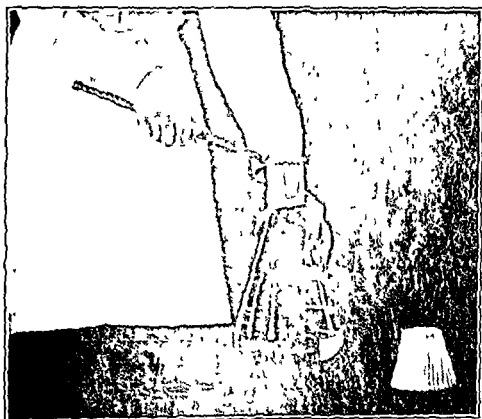


FIG. 1.

Stock and Standard solutions are mixed by drawing air through them, using a filter pump, water jet form (marketed by A. H. Thomas Co.) The air is washed by drawing it through soda lime, a weak sulphuric acid solution and finally distilled water before it enters the solution it is to mix. An empty flask is placed between the suction pump and the solution being mixed, as a precaution against the filter pump "backfiring."

Stock and Standard saline solutions are filtered by means of sintered glass filters, no paper filters are used in this laboratory. A sintered glass filter consists of a disk of porous glass sealed in the mouth of a funnel. The filter is immersed in the solution to be filtered, the end of the neck of the filter is connected by means of rubber tubing to a perforated rubber stopper in the mouth of an empty carboy in which a vacuum is created by means of the filter pump, the filtrate being drawn through the neck of the filter, the rubber tubing and into the empty carboy.

carded. If less than 15 cc were used, the thiocyanate solution is too concentrated and must be diluted using the formula

$$\frac{\text{Volume Ammon thio}}{\text{Titer}} \times (15 - \text{Titer}) = \text{cc of distilled water to be added}$$

If the burette reading was 13.5 and the total remaining volume of Stock ammonium thiocyanate solution is 972 cc then the calculation is

$$\frac{972}{13.5} \times (15 - 13.5) = 108 \text{ cc of distilled water to be added to the}$$

Stock ammonium thiocyanate solution to bring it to the right concentration. After the addition of the water the solution is mixed and again titrated.

If the reading of the burette was greater than 15 cc, then the Stock ammonium thiocyanate solution is weak in concentration and some ammonium thiocyanate must be added using the formula

$$\frac{\text{Volume Ammon thio}}{\text{Titer}} \times (\text{Titer} - 15) \times 0.18 = \text{gm of ammonium thiocyanate to be added}$$

If the burette reading was 15.5 cc and the total remaining volume of Stock ammonium thiocyanate solution is 961 cc then the calculation is

$$\frac{961}{15.5} \times (15.5 - 15) \times 0.18 = .08 \text{ gm ammonium thiocyanate to be added}$$

Having added the chemical the solution is mixed and again titrated. The figure 0.18 is obtained from the formula 180 gm of ammonium thiocyanate in 1,000 cc or 0.18 gm per cc.

All the reagents are now ready to titrate the Stock saline solution. Into an Erlenmeyer flask of about 250 cc capacity, 10 cc of Stock solution (saline) and 15 cc of Stock silver nitrate solution are carefully pipetted, 15 cc of concentrated nitric acid and 0.3 gm of powdered ferric ammonium sulphate are added. The flask is shaken and allowed to stand 5 minutes in a dark place, then its contents are titrated with the Stock ammonium thiocyanate solution. Ten cubic centimeters of Stock saline solution should combine chemically with 10 cc Stock silver nitrate solution, therefore requiring 5 cc of the Stock ammonium thiocyanate solution to bring the titration to the proper end point. The contents of the flask are discarded. If less than 5 cc of the thiocyanate solution were used the Stock saline solution is of too strong a concentration and is diluted, using the formula

$$\frac{\text{Volume Stock Saline Sol}}{10} \times (5 - \text{Titer}) = \text{cc of distilled water to be added to the Stock saline solution}$$

If the reading of the burette containing the Stock ammonium thiocyanate solution were 4 cc then the 10 cc of Stock saline solution contained enough sodium chloride to combine with 11 cc of the Stock silver nitrate solution. Therefore every 10 cc of Stock saline solution must be diluted with distilled water to 11 cc or applying the formula and assuming that the total volume of Stock saline solution is 9,980 cc

$$\frac{9980}{10} \times (5 - 4) = 998 \text{ cc of distilled water to be added to the Stock saline solution}$$

After addition of the water the solution is again mixed and titrated, e.g. If the reading of the burette containing the Stock ammonium thiocyanate solution were 6 cc, then the Stock saline solution is weak in concentration and must be strengthened with sodium chloride, using the formula.

$$\frac{\text{Volume Ammon Thio}}{\text{Titer}} \times (15 - \text{liter}) = \text{cc distilled water to be added to Standard ammonium thiocyanate solution}$$

After addition of distilled water the solution is mixed and again titrated. If more than 15 cc of thiocyanate solution were used the solution is weak in concentration and ammonium thiocyanate chemical must be added according to the formula

$$\frac{\text{Volume Ammon Thio}}{\text{Titer}} \times (\text{Titer} - 15) \times 0.0112 = \text{gm ammonium thiocyanate to be added to the Standard ammonium thiocyanate solution}$$

These "Standard" reagents are now ready for use and are stable for approximately several months.

To determine the percentage content of the Standard saline solution, 20 cc are pipetted into a 250 cc Erlenmeyer flask to which are added 25 cc of Standard silver nitrate solution, 15 cc of concentrated nitric acid and 0.3 gm of powdered ferric ammonium sulphate. The mixture is shaken, allowed to stand in a dark place for five minutes and then titrated with Standard ammonium thiocyanate solution to a salmon pink end point. If the reading of the burette is 5 cc then the saline is of the correct concentration. The contents of the flask are discarded. If less than 5 cc of the thiocyanate solution were used, then the saline solution is strong in concentration and must be diluted with water to the formula

$$\frac{\text{Total Volume of Saline}}{20} \times (5 - \text{liter}) = \text{cc of distilled water to be added to the Standard saline solution}$$

If more than 5 cc of Standard ammonium thiocyanate solution were used then the Standard saline solution is weak in concentration of sodium chloride and more Stock saline solution must be added according to the formula

$$\frac{\text{Total Volume of Saline}}{20} \times \frac{(\text{Titer} - 5)}{15} = \text{cc of Stock saline solution to be added to the Standard saline solution}$$

If it has been necessary to make any adjustment to the Standard saline solution it is mixed by drawing air through it and again titrated to make certain that it is of the correct concentration.

#### BOTTLING THE STANDARD SALINE SOLUTION

The Standard saline solution is bottled by siphoning it into volumetric flask of desired size and then pouring the contents into a permanently labelled flask of twice the volume, which has been thoroughly washed with hot water and soap, rinsed with tap water and finally with distilled water. The flasks are capped immediately in order that no dust particles may enter. Each cap is stamped with the date of manufacture. The caps are held in place by means of two wire loops, one at the upper and one at the lower part of the skirt of the cap. The loops are tightened by means of a hook on the end of a twisted rod on which a nut, in the form of a handle, screws. The operator holds the handle and pulls, thereby turning the rod and twisting the wire loop. The procedure of tightening the wire loops requires a few seconds of time. Each flask is examined before it leaves the laboratory for the sterilization room where it is autoclaved promptly.